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VETERINARY HEMATOLOGY

OSCAR W. SCHALM

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VETERINARY HEMATOLOGY

By

OSCAR W. SCHALM, D.V.M., PH.D.

*Professor of Clinical Pathology, University of California School of Veterinary Medicine,
Agricultural Experiment Station, Davis, California*

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Preface

INTEREST in hematology as an aid to diagnosis is increasing in the practice of veterinary medicine. A need exists for a textbook in the English language on the subject to serve the student, the practitioner, and the research worker in animal diseases. I hope that the present volume will fill this need in part and that the publication of this text will stimulate an even greater interest in the subject.

The information gained from the examination of blood morphology in diseased animals is not in itself diagnostic, except in a few special instances, but such information taken together with the physical examination and medical history should provide an excellent basis for judgment relative to (1) the nature of the disease, (2) the extent of injury to the tissues and organs of the body and (3) the responses of the defense mechanisms of the patient. This more complete view of the patient and its disease should lead to a more selective program of treatment and increase the accuracy of prognosis.

Considerable information is available with respect to the normal blood morphology of the common domestic animals, but such data are widely scattered in the medical literature and not readily available for classroom use or ease of reference in practice. There is considerably less information available in the literature on changes in the blood picture in disease and especially with respect to the differences among the domestic animals in the responses of their hematopoietic tissues to the stress of disease.

The subject matter of this volume is based upon (1) a search of the English language medical literature for published data on the blood morphology in the common domestic mammals, and (2) original data on the blood of the dog, cat, cow, sheep, goat, horse and pig in health and disease obtained from the examination of approximately 30,000 blood samples in the Department of Clinical Pathology, School of Veterinary Medicine, University of California between the years 1950 and 1960.

Blood volume is discussed from the point of view of the ratio of cells to plasma in the various parts of the vascular system and special attention is directed to the importance of viewing the data obtained from blood examination in light of the water balance of the animal. Sick animals often are found to be showing varying degrees of dehydration which leads to hemoconcentration and produces falsely elevated values for erythrocyte and leukocyte numbers and hemoglobin concentration. The existence of anemia may be masked by hemoconcentration and the erythrocyte sedimentation rate may be retarded.

The technics for blood study that are described and discussed have been selected either because they are used widely and/or because they are especially applicable to the practice of veterinary medicine. No attempt has been made to include every method for blood examination. The hematocrit and the stained blood film are given special attention for this combination of tests is notably suited to routine use in veterinary practice. The erythrocyte sedimentation rate is a useful tool especially in canine practice, and to assist in interpretation a table is included showing the distance fall of the red cell mass in one hour to be anticipated for each unit of erythrocyte volume between 9 and 50 per cent in the Wintrobe hematocrit tube.

The nomenclature of blood cells and their precursors and cell variants in disease is that recommended by the committee sponsored by the American Society of Clinical Pathologists and the American Medical Association. Some slight modifications and additions to the committee's recommendations were necessary to meet the special needs of veterinary hematology. Ability to interpret the cellular patterns in peripheral blood in disease depends upon knowledge of the maturation of the erythrocytes and leukocytes in bone marrow and other hematopoietic tissues. Brief reference is made to the development of blood cells in the embryo and fetus, and photographs are presented of the maturation series of erythrocytes and granulocytes in the post-natal bone marrow. In addition, morphologic descriptions accompanied by photographs are given of the erythrocytes, leukocytes and thrombocytes of peripheral blood of the dog, cat, cow, sheep, horse and pig.

The erythrocytic series is discussed in detail relative to formation, function, life span, and destruction. The intent is to provide an adequate basis for interpretation of the blood pictures in the anemias. Knowledge of erythrocyte morphology as influenced by disease is most useful in formulating a rational attitude toward the problem of anemia. Fortified with such knowledge, the practitioner will employ hematinics and/or blood transfusions judiciously and in many cases of anemia he will have the conviction that neither is indicated in the treatment of the patient.

The leukocytes are considered from the point of view of variations in response to disease to be found among the different domestic animals. It is not proper to apply the same yardstick in interpretation of the leukocyte blood pictures in disease in all animal types. In the cow, the initial response to bacterial infection is quite frequently a frank leukopenia, and later when a leukocytosis develops it is limited in magnitude and seldom exceeds twice the normal maximum total leukocyte count. The dog and cat, on the other hand, respond dramatically to localized bacterial infection and their total leukocyte counts often reach 50,000 to 100,000 per mm.³ of blood. The discussion of the leukocytes includes a review of the leukemia complex and points to some differences between the

leukemias of man and animals. The significance of lymphosarcoma in the bovine as a herd problem is discussed in light of original data.

The concluding chapter and an Appendix of 27 selected cases present original data on the characteristic blood changes in some common diseases of domestic animals. Hemograms of cases from the Veterinary School Clinic are presented for chronic interstitial nephritis, pyometra, hypothyroidism, infectious hepatitis, distemper, and leptospirosis in the canine, panleukopenia in the feline, traumatic reticulitis, acute mastitis, and retained placenta in the bovine, and the cases included in the Appendix were selected to serve as reference material throughout the various chapters so as to demonstrate more clearly a point under discussion.

I take pleasure in expressing my gratitude to all who have helped to make this book possible. I am especially grateful to the staff of the Veterinary Clinic of the University of California for supplying blood and other materials from interesting cases, in many instances at daily intervals so that the dynamics of hematopoiesis in disease could be better visualized. Special recognition is due Mrs. Virginia Gilmore for the vast amount of effort put forth in the application of her technical skills in the collection of original data presented in the numerous tables included in this volume. I make mention, also, of Dr. Otto Straub for his assistance in the accumulation of normal blood values in both growing and adult swine. Without his effort, no original data on the blood of swine could have been included. Finally, I express my gratitude to the staff of Messrs. Lea and Febiger for encouragement to undertake the task of writing the manuscript and for their helpfulness and skill in preparing it for publication.

O. W. SCHALM

Davis, California

Contents

CHAPTER 1

BLOOD VOLUME AND WATER BALANCE

Blood Volume	15
Plasma Volume Measurement	17
Trapped Plasma in the Venous Hematocrit	18
Erythrocyte Volume Measurement	19
The Ratio of Body to Venous Hematocrit	20
Blood Volume in Animals	21
Blood Studies in Relation to Water Balance	22
Hemorrhage and Blood Restoration	24

CHAPTER 2

MATERIALS AND METHODS FOR THE STUDY OF THE BLOOD

The Microscope	30
The Lenses	30
Obtaining Correct Illumination	31
Cleaning Glassware	32
Collecting and Handling Blood for Laboratory Study	33
Anticoagulants	34
Obtaining the Blood Sample	37
Handling of the Blood	38
The Blood Film	40
Slide Method	41
Cover-slip Method	43
Basic Principles for Staining Blood	43
Materials and Methods Used in Staining the Blood Film	44
Standard Wright's Stain	45
Modified Wright's Stain	45
Staining Blood Films on Slides by Wright's Method	45
Modifications for Staining Slides with Wright's Stain	46
Staining Blood Films on Slides in Coplin Jars	46
Directions for Staining Blood Contained on Cover-slips	46
Wright's-Leishman Stain	47
Giemsa's Stain	48
Staining and Counting Reticulocytes	48
Causes and/or Correction of Poor Staining	48

Microscopic Examination of the Blood Film	49
Blood Examination by Wet Film Method	51
The Wintrobe Hematocrit Method	54
Sedimentation Rate of Erythrocytes	55
Detection of Reticulocytosis by Sedimentation Test	57
The Packed Cell Volume	59
The Buffy Coat	59
The Plasma Compartment	60
The Microhematocrit	61
Determination of Hemoglobin	64
Direct Matching	66
Hematin Methods	66
Oxyhemoglobin, Direct Method	66
Photoelectric Colorimeter and Spectrophotometric Methods	66
Enumeration of Erythrocytes and Leukocytes in the Hemocytometer	68
Filling the Pipette	68
The Counting Chamber	70
Counting the Cells and Calculations	71
Inherent Errors in the Blood Cell Count as Made with the Hemocytometer	71
Enumeration of Eosinophil Leukocytes	73
Measurement of Mean Erythrocyte Size	74
Direct Measurement of Erythrocyte Diameter	74
Diffraction Methods for Obtaining Mean Diameter of the Erythrocyte	74
The Wintrobe Erythrocytic Indexes	76
Mean Corpuscular Volume	76
Mean Corpuscular Hemoglobin	77
Mean Corpuscular Hemoglobin Concentration	77
Aspiration of Bone Marrow from the Iliac Crest	77
Rapid Tests for Hemostatic Defects	79
Bleeding Time	80
Coagulation Time	80
Platelet Estimations	80
Clot Retraction or Syneresis	81
Blood Groups and Cross Matching	81
Examination for Microfilaria in Canine Blood	83
Erythrocyte Fragility Test	84

CHAPTER 3

STANDARDIZATION OF NOMENCLATURE OF CELLS AND
DISEASES OF THE BLOOD AND BLOOD FORMING ORGANS.
CHARACTERIZATION OF IMMATURE AND MATURE
BLOOD CELLS

Recommended Spelling and Definition of Terms	91
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Fundamentals of Terminology	92
Terminology for Use with Cell Variants or Diseases of the Blood	93
Recommended Terms and Description of Individual Cells	99
Bone Marrow Evaluation	101
The Myeloid:Erythroid (M:E) Ratio	107
Description of Cells of Peripheral Blood	107
Canine Blood Cells	107
Feline Blood Cells	112
Bovine Blood Cells	115
Ovine Blood Cells	121
Equine Blood Cells	122
Porcine Blood Cells	125

CHAPTER 4

NORMAL VALUES IN BLOOD MORPHOLOGY

General Considerations	132
The Dog	134
Erythrocyte Number and Hemoglobin Values	135
Sex Differences	135
Erythrocyte Diameter	135
Reticulocytes	135
Sedimentation of Erythrocytes	139
Erythrocyte Fragility	140
Leukocyte Counts, Total and Differential	141
The Myeloid-Erythroid Ratio	141
Thrombocytes	142
Icterus Index	142
Specific Gravity	142
The Cat	143
Erythrocyte Number and Hemoglobin Values	147
Sex Differences	148
Erythrocyte Diameter	148
Reticulocytes	148
Howell-Jolly Bodies	148
Sedimentation of Erythrocytes	148
Erythrocyte Fragility	148
Leukocyte Counts, Total and Differential	149
The Myeloid-Erythroid Ratio	149
Thrombocytes	150
Icterus Index	150
Specific Gravity	150
The Cow	150
Effects of Age Upon Mean Blood Values of Cattle	151

The Red Cells in Young Bovines	152
The PCV and Hemoglobin in Young Bovines	153
The Leukocytes in Young Bovines	153
Erythrocytic Values in Adult Cattle	156
Erythrocyte Number, PCV and Hemoglobin	156
Influence of Sex	156
Breed Differences	158
Influence of Climate and Nutrition	158
Effect of Parturition	160
Erythrocyte Diameter	160
Reticulocytes	160
Sedimentation of Erythrocytes	161
Erythrocyte Fragility	161
Leukocytic Values in Adult Cattle	162
Leukocyte Counts, Total and Differential	162
Occurrence of Young Neutrophils	162
Effect of Estrus	164
Effect of Pregnancy	164
Effect of Parturition	164
Effect of Drying-off of Lactation	164
The Myeloid-Erythroid Ratio	165
Thrombocytes	165
Icterus Index	166
Specific Gravity	166
The Sheep	166
Effect of Age on Blood Values	167
Erythrocyte, PCV and Hemoglobin Values in Adult Sheep	172
Influence of Sex and Breed	172
Erythrocyte Diameter	172
Reticulocytes	173
Sedimentation of Erythrocytes	173
Erythrocyte Fragility	173
Leukocyte Counts, Total and Differential	173
The Myeloid-Erythroid Ratio	173
Thrombocytes	173
Icterus Index	174
Specific Gravity	174
The Goat	174
Erythrocytes, PCV and Hemoglobin	174
Erythrocyte Diameter	174
Reticulocytes	175
Sedimentation of Erythrocytes	175
Erythrocyte Fragility	175
Leukocyte Counts, Total and Differential	175

The Myeloid-Erythroid Ratio	175
The Horse	177
Erythrocyte Number, PCV and Hemoglobin	178
Influence of Age on Erythrocytes	178
Influence of Sex, Pregnancy, Lactation, Barrenness	179
Influence of Excitement	179
Effect of Internal Parasites on Erythrocytic Values	179
Erythrocyte Diameter	179
Reticulocytes	180
Sedimentation of Erythrocytes	180
Erythrocyte Fragility	181
Leukocyte Counts, Total and Differential	181
The Myeloid-Erythroid Ratio	181
Thrombocytes	185
Icterus Index	185
Specific Gravity	186
Blood Values of the Donkey and Mule	186
Erythrocytes	186
Leukocytes	186
The Pig	188
The Problem of Adequate Iron for Suckling Pigs	188
The Hematology of Young Pigs	189
Porcine Hematology in General	191
Erythrocyte Number	191
Erythrocyte Size	191
Reticulocytes	191
Blood Volume and Volume Per Cent Erythrocytes	194
Hemoglobin	194
Erythrocyte Life Span	194
Erythrocyte Fragility	194
Sedimentation Rate of Erythrocytes	195
Leukocyte Counts, Total and Differential	195
The Myeloid-Erythroid Ratio	195
Thrombocytes	196
Icterus Index	196
Specific Gravity	196

CHAPTER 5

HEMATOPOIESIS

Hematopoiesis in the Embryo and Early Post-natal Life	205
Hepatic Hematopoiesis	205
Hematopoiesis in the Spleen and Lymph Nodes	206
The Pre-natal Bone Marrow	207

Contents

11

Embryonic Hematopoiesis Related to the Post-natal State	207
The Hematopoietic System in Growing and Adult Animals	207
Bone Marrow Sections	208
Bone Marrow Aspirations	210
Bone Marrow Volume	211
Bone Marrow Transplants	211
The Lymph Nodes and Follicles	212
The Liver	212
The Spleen	213
The Stomach	214
The Reticulo-endothelial System	215
The Kidneys	215

CHAPTER 6

THE ERYTHROCYTES: THEIR PRODUCTION, FUNCTION AND DESTRUCTION

The Fundamental Stimulus for Erythropoiesis	218
The Reticulocyte	220
Essential Materials for Erythrocyte Production	221
Structure and Function of the Erythrocyte	223
Hemoglobin Synthesis and Destruction	224
Hemoglobin Synthesis	224
Hemoglobin Destruction	226
Erythrocyte Life Span	227
Destruction of Erythrocytes	229

CHAPTER 7

THE ERYTHROCYTES IN DISEASE

Polycythemia Vera	236
The Sedimentation Rate of Erythrocytes	237
The Anemias	239
Blood Transfusion	239
Compensatory Adjustments in Anemia	240
Clinical Signs in Anemia	240
Morphologic Classification of the Anemias and Their Treatment	242
Macrocytic Anemias	242
Normocytic Anemias	242
Microcytic Hypochromic Anemias	242
The Etiologic Classification of the Anemias	243
Blood Morphology in the Anemias	244
Bone Marrow Response in Acute Blood Loss or Destruction	244

Signs of Abnormal Erythrogenesis or Bone Marrow Dysplasia	245
Anemias Associated with Nutritional Deficiencies	249
Vitamin Deficiencies	249
Mineral Deficiencies	253
Some Hemolytic Anemias with Hemoglobinuria	254
Copper Poisoning	254
Post-parturient Hemoglobinuria in Cattle	255
Hemoglobinuria in Calves from Water Intoxication	255
Bacillary Hemoglobinuria in Cattle and Sheep	255
Leptospirosis	256
Hemolytic Anemia of the Newborn Due to Isoimmunization	257
Some Hemolytic Anemias without Hemoglobinuria	257
Anaplasmosis	257
Feline Infectious Anemia	258
Anemia Associated with Hypersplenism	259
Examples of Hypoplastic Anemia	259
Bracken Fern Poisoning	260
Trichloroethylene-extracted Soy Bean Oil Meal Poisoning	260
The Radiation Syndrome	260
Blood Loss Associated with Parasitism	262
Secondary Anemias from Erythrocytic Hypoplasia	263
Nephritis with Uremia	263
Neoplasia	263
Hypothyroidism	264
Parasitism in Sheep and Cattle	264

CHAPTER 8

THE LEUKOCYTES

Brief Summary of the Origin and Functions of Leukocytes	269
Hormonal Influence on Leukocytes	270
Effectiveness of Granulopoiesis in Disease	271
Species Variations in Total Leukocyte Response in Disease	271
Classification of the Total and Differential Leukocyte Response	272
Regenerative Left Shift	272
Degenerative Left Shift	272
Leukemoid Blood Picture	272
Absolute and Relative Leukocyte Numbers	272
Leukocyte Response in Relation to the Type of Disease Process	273
Interpretation of the Leukocyte Picture	274
Dynamics of Granulopoiesis	274
The Biology of Inflammation	276
The Lymphocyte	278

CHAPTER 9

THE LEUKEMIA COMPLEX

Comparison of the Leukemias of Man and Animals	281
Facts and Theories Regarding the Etiology of Leukemia	283
Transmissible Agent	283
Genetic Influence	283
Ionizing Radiation	284
The Leukemia Complex in the Bovine	285
Clinical Signs and Differential Diagnosis	286
Lymphosarcoma Herds and Their Detection	290
The Leukemia Complex in the Canine	293
Clinical Signs of Malignant Lymphoma	293
Granulocytic Leukemia	298
Monocytic Leukemia	299
Myelomatosis	300
The Leukemia Complex in the Feline	301
Erythroleukemia	301
Basophilic Leukemia	303
The Leukemia Complex in the Equine	304
Myelomatosis	305
Miscellaneous	307
Lymphosarcoma in the Goat	307
Leukemia Complex in Swine and Sheep	308

CHAPTER 10

BLOOD PICTURES IN SOME COMMON DISEASES
OF DOMESTIC ANIMALS

Chronic Interstitial Nephritis with Uremia in the Canine	312
Pyometra in the Canine	316
Hypothyroidism in the Canine	316
Leptospirosis in the Canine	319
Canine Distemper	320
Infectious Canine Hepatitis	322
Infectious Feline Panleukopenia	324
Traumatic Gastritis or Reticulitis in the Bovine	326
Acute Bovine Mastitis	328
Effect of Parturition and Retention of Fetal Membranes	330

APPENDIX

Case 1. Peritonitis from Gunshot Wound in the Canine	335
Case 2. Kidney Abscess in the Canine	336

Case 3.	Cellulitis in the Canine Associated with Anemia of Infection	337
Case 4.	Pyometra in the Canine	338
Case 5.	Malignancy in the Equine	339
Case 6.	Malignancy in the Canine	340
Case 7.	Multiple Abscesses in the Equine with Associated Anemia of Chronic Infection	341
Case 8.	Traumatic Reticulitis in the Bovine	342
Case 9.	Leptospirosis in the Canine	343
Case 10.	Chronic Interstitial Nephritis in the Canine	343
Case 11.	Chronic Interstitial Nephritis with Uremia in the Canine	344
Case 12.	Hypothyroidism in the Canine	345
Case 13.	Blood Loss Anemia in the Equine	347
Case 14.	Hemolytic Crisis in the Canine	347
Case 15.	Chronic Blood Loss in the Canine	349
Case 16.	Megaloblastic Anemia in the Canine	350
Case 17.	The Hematology of Anaplasmosis	353
Case 18.	Haemonchosis in the Ovine	353
Case 19.	Trichostrongylidosis in the Bovine	354
Case 20.	Equine Infectious Anemia	355
Case 21.	Symptomatic Acquired Hemolytic Anemia in the Canine	356
Case 22.	Chronic Lead Poisoning in the Canine	360
Case 23.	Congenital Porphyria (Pink Tooth) in the Bovine	361
Case 24.	Anemia Associated with Capillary Fragility in the Canine. Possible Vitamin C Deficiency	362
Case 25.	Iron Deficiency Anemia Due to Chronic Blood Loss in the Canine from Heavy Infestation with Stick-tight-Fleas	364
Case 26.	Anemia Associated with Hypersplenism in the Canine	365
Case 27.	Necrobacillosis and Hemorrhage from Abomasal Ulcers in the Bovine	366

Veterinary Hematology

Chapter 1

Blood Volume and Water Balance

ALL animal cells, from the free-living protozoan to the specialized tissue cell of the higher forms of animal life, require water to carry on their functions. The free-living, single-celled protozoan lives in fresh water or in the sea and it derives its food therefrom and discharges its wastes therein. In the more complex forms of animal life, the blood serves the purpose of supplying each cell with the required water, oxygen, electrolytes, nutrients, and hormones and the blood receives the waste products of metabolism for transport to the organs of excretion.

BLOOD VOLUME

The total volume of circulating blood is a function of lean body weight. The volume of blood is so important to the dynamics of circulation that it is kept remarkably constant despite (*a*) the periodic intake of water, (*b*) the production of water of metabolism and (*c*) the continuous water loss *via* the skin, lungs, kidneys, mammary glands, and alimentary tract. Even in instances of sudden loss of blood by hemorrhage, the replacement of the lost volume by movement of interstitial fluid into the vascular system is initiated within minutes and continues until the original volume is restored within hours. The depleted erythrocyte volume requires a much longer period for restoration for the addition of newly formed erythrocytes to the blood begins not before 72 hours and continues for days and even weeks depending upon the magnitude of the loss. The plasma volume serves as a buffer to maintain blood volume relatively constant and as the circulating red cell volume is gradually restored, the plasma volume is correspondingly reduced. Even the addition of blood by transfusion does not alter the total volume of blood for long for the plasma becomes reduced to accommodate the additional cell volume introduced by the transfusion.

The earliest method for estimating blood volume consisted of bleeding the animal to death followed by washing-out the blood vessels and adding the blood contained in the washings to that collected during

bleeding. Blood volume was estimated from the dilution of one of the measurable blood constituents as compared to its concentration in the blood of the same animal collected prior to the experiment. Another early method consisted of the injection of a known quantity of isotonic NaCl into the vascular system and shortly thereafter noting the extent of dilution of the blood as determined by the change in specific gravity, red cell number or hemoglobin concentration. Results in man and animals with such methods were summarized in 1909 by Reichert and Brown⁴ and in 1921 by Erlanger.² Examples from these early reviews are included in Table 1 for historical interest.

Table 1. Examples of Blood Volume Measurements in Several Domestic Animals as Reported in the Literature

<i>Animal</i>	<i>Reference</i>	<i>Method Employed</i> <i>Plasma</i>	<i>Red Cells</i>	<i>Ml. of Blood</i> <i>per kg. body wt.</i> <i>(mean value)</i>	<i>Remarks</i>
Dog	Reichert ⁴	Wash-out method	—	72.0	Prior to 1909
	Erlanger ²	Wash-out, etc.	—	77.0	Mean of 9 reports prior to 1920
	McQuarrie ¹⁹	Acacia or gelatin	—	97.6	
	Dawson ¹⁷	T-1824	—	92.0	
	Smith ¹⁶	T-1824	Carbon monoxide	92.0	Includes 2.0 ml. esti- mated for leuko- cytes.
	Courtice ³⁰	T-1824	—	79.0	29 mongrel dogs
	Courtice ³⁰	T-1824	—	114.0	4 greyhounds
	Billings ²⁷	T-1824	—	91.0	Correction factor of 0.95 applied to the venous hematocrit. (10 dogs.)
	Reeve ⁵⁴	T-1824	P ³²	94.4	8 dogs
	Clark ²⁹	T-1824	Cr ⁵¹	81.0	41 dogs
	Clark ²⁹	T-1824	—	87.2	39 dogs
	Clark ²⁹	—	Cr ⁵¹	70.7	17 dogs
Cat	Reichert ⁴	Wash-out method	—	65.0	Prior to 1909
	Erlanger ²	Washout and other early methods	—	69.0	Prior to 1920
Cow	Reichert ⁴	Wash-out method	—	77.0	Prior to 1909
	Turner ³³	Vital Red	—	62.5	54 growing dairy ani- mals.
	Turner ³³	Vital Red	—	63.8	24 non-lactating cows
	Turner ³⁸	Vital Red	—	81.1	41 lactating cows.
	Miller ³³	Brilliant vital red	—	59.5	Age or mammary function not given. (105 determination 17 animals.)
	Reynolds ³⁵	T-1824	—	57.0	0.94 correction to the venous hematocrit (non-lactating.)
	Stahl ³⁷	T-1824	—	105.6	17 young dairy calves weighing 80 kg. ±
	Stahl ³⁷	—	Cr ⁵¹	78.2	

Sheep	Reichert ⁴	Washout method	—	80.0	Prior to 1909
	Barcroft ²⁶	T-1824	—	58 to 64	3 hysterectomized sheep
	Schambye ²⁶	T-1824	P ³²	58.0	6 sheep
Goat	Reichert ⁴	Washout methods	—	62.0	Prior to 1909
	Courtice ³⁰	T-1824	—	70.0	30 animals
Pig	Bush ²⁸	Fe ⁵⁹	P ³²	95.0	at 10 kg. body wt.
		"	"	83.5	" 20 " " "
		"	"	78.3	" 30 " " "
		"	"	73.0	" 40 " " "
		"	"	69.4	" 50 " " "
		"	"	66.2	" 60 " " "
		"	"	63.6	" 70 " " "
		"	"	61.5	" 80 " " "
		"	"	59.7	" 90 " " "
		"	"	58.0	" 100 " " "
		"	"	56.5	" 110 " " "
Horse	Reichert ⁴	Wash-out method	—	97.0	Prior to 1909
	Courtice ³⁰	T-1824	—	72.0	2 animals
	Cronin ³¹	T-1824	—	81.0	1 animal
	Julian ³²	—	P ³²	109.6	6 "hot-blooded" animals.
	"	—	"	126.0	Same fat-free body wt.
	"	—	"	71.7	4 Percherons
	"	—	"	94.9	Same animals but calculated as fat-free body-weight.

Plasma Volume Measurement

About 1920, methods for estimating blood volume by the injection of dyes or other substances into the blood stream were coming into use, and were based upon measuring the dilution of a known quantity of the injected material. A satisfactory tracer for this purpose must be non-toxic and it must remain in the vascular system, without loss, for a sufficient length of time to permit complete mixing with the blood and the removal of blood samples for the dilution studies. Colloids, such as acacia or gelatin were tried,¹⁹ but certain dyes proved to be more suited to the purpose and the dye dilution method has received much study. Vital red and brilliant vital red were used at first but they were soon replaced by the blue azo dye, T-1824, commonly known as Evan's blue.¹⁷ One advantage of the blue color is that when hemolysis is present it is more readily observed than is the case with red dyes. This is an important factor in the accurate measurement of plasma volume by the dye dilution method.

The dye T-1824 binds to the albumin fraction²⁰ of blood plasma and, therefore, it is a method for the direct measurement of plasma volume. In recent years, it has been possible to tag plasma by other methods, such as with antigens¹⁸ or radioactive serum albumin tagged with iodine^{131, 21, 22}. The simultaneous use of T-1824 and antigens or I¹³¹

albumin for plasma volume measurement has shown good agreement among results obtained and point to the validity of T-1824 measurements of plasma volume when carefully done. The criticisms that have been directed at the T-1824 dye dilution method apply as well to other tagged protein methods for plasma volume measurements.²¹

In using the dye dilution method, oxalated or heparinized venous blood is centrifuged at 3,000 r.p.m. for 30 minutes or longer in a calibrated tube to produce a separation of the cells and plasma. The result is designated the venous hematocrit and the percentages of plasma and cells in the blood sample can be determined directly from the calibration of the tube. The values for cells and plasma are expressed in volume per cent and the term hematocrit has come to mean specifically the volume per cent of erythrocytes in the sample after centrifugation. To measure dilution of the dye in the circulation the plasma is removed from the centrifuged blood sample and the quantity of dye/ml. contained therein is measured by means of a colorimeter or spectrophotometer. The plasma volume and total blood volume are calculated as follows:

$$\text{Volume of plasma in ml.} = \frac{\text{mg. dye injected}}{\text{mg./ml. in plasma of venous blood.}}$$

$$\text{Blood volume in ml.} = \frac{\text{Plasma volume}}{100 - \text{volume per cent cells in the venous hematocrit.}} \times 100$$

Trapped Plasma in the Venous Hematocrit

Blood volume calculated from the plasma volume has been shown to be about 4 per cent less than the true volume because the routine hematocrit generally does not result in complete separation of erythrocytes and plasma and as a consequence some plasma remains in the red cell column; this plasma has been designated trapped plasma^{6,8} Hematocrits of human blood ranging from 38.4 to 53.6 per cent erythrocytes were shown, by use of iodinated-albumin containing the isotope I^{131} , to have a mean error of 3.79 per cent of the centrifuged cell value due to trapped plasma.¹⁰ The literature on the problem of trapped plasma suggests correction factors varying from 2.25 to 8.5 per cent to be applied to the centrifuged red cell value.⁷ A correction factor of 5.0 per cent has been applied to the venous hematocrit of the dog²⁷ and sheep³⁶ and a 6.0 per cent correction factor has been used to correct the venous hematocrit of the cow.³⁵ However, there is reason to caution against the acceptance of a single correction factor for all hematocrit readings of a given species of animal by reason of the fact that the volume of trapped plasma varies with the length of the red cell column, the extent of rouleau formation or erythrocyte agglutination in disease, and the size of the erythrocytes.⁵ This can be explained by the well known fact that centrifugal force

increases as the radius increases and thus the force applied to the cells in the hematocrit tube varies with the position of the cells in the tube; the volume of trapped plasma increases from the bottom upward and the overall percentage of trapped plasma is greatest with large hematocrit values. Tighter packing of cells is enhanced by the presence of erythrocyte rouleaux or agglutination and by large size of erythrocytes. The volume of trapped plasma in the venous hematocrit of animal bloods will be influenced by special characteristics of the erythrocytes of the animal in question. For example, erythrocyte rouleau formation is normally a pronounced feature in horse blood but it is essentially absent in cow, sheep and goat bloods; also, erythrocyte size is variable, the canine erythrocyte will average 3 times the mean corpuscular volume of the goat red cell. The amount of trapped plasma can be reduced slightly by increasing the time of centrifugation at 3,000 r.p.m. beyond the routine 30 minute period,⁵ also, the trapping of plasma can be minimized by increasing the r.p.m. as is done in the microhematocrit. Goat blood centrifuged at $2,000 \times$ gravity for 30 minutes (standard Wintrobe hematocrit method) was shown by plasma isotope tag to have up to 20 per cent of the red cell column consisting of plasma; the same blood centrifuged in capillary tubes at 15,000g for 7.5 minutes had only 2 per cent of plasma in the red cell column.⁹ It is advisable to extend the period of centrifugation of the standard hematocrit to 45 minutes or 1 hour when animal bloods, such as cow, sheep, and goat, are under study.

Erythrocyte Volume Measurement

Development of the isotope method for biological tracers brought new tools for the measurement of plasma volume, cell volume and total blood volume. Radioactive iron, Fe^{55} and Fe^{59} , radioactive phosphorus, P^{32} , and more recently radioactive chromium, Cr^{51} , have been used to tag erythrocytes for direct measurement of total red cell mass or volume. The use of serum albumin labeled with I^{131} for the direct measurement of plasma volume has been mentioned above.

Tagging of erythrocytes with radioactive iron is accomplished by administering the isotope to the donor animal for several weeks during which time the isotope becomes incorporated within the hemoglobin molecule of newly formed red cells. Such a tag is permanent for the life span of the cell. Blood is removed from the donor and injected into the experimental animal²⁴ and the total red cell volume is determined by measuring the dilution of the tagged erythrocytes after complete mixing in the circulation. Radioactive phosphorus, P^{32} , is easier to use for the tagging process is accomplished *in vitro* and thus blood is withdrawn from the experimental animal, the erythrocytes are tagged with P^{32} , and the blood is reinjected into the same animal. A disadvantage is that

P^{32} exchanges rapidly between cells and plasma causing the radioactivity of the erythrocytes to fall significantly after 1 to 3 hours.²³ Radioactive chromium, Cr^{51} , tags erythrocytes *in vitro* by attaching to the globin fraction of the hemoglobin molecule. It has a distinct advantage over P^{32} in that the radioactivity is retained by the erythrocytes without significant loss for up to 24 hours.²⁵

The Ratio of Body to Venous Hematocrit

Direct measurement of total red cell volume by means of tagged-erythrocytes reveals that the circulating red cell volume or body hematocrit is considerably less than the value calculated from the data on plasma volume and the venous hematocrit.²⁴ As early as 1920,¹⁶ significant differences were noted between red cell volume calculated from plasma volume and venous hematocrit and the value determined by direct measurement with a carbon monoxide tag.¹⁶ The explanation given at that time is now a proven fact and that is that the venous hematocrit is in error because the ratio of cells to plasma is not uniform throughout the vascular system. Blood in the small vessels of the body has a significantly lower hematocrit value than blood in the heart or large vessels of the body. This is explained on the basis that red cells flow in an axial stream in small vessels and pass through the vessels more quickly than the plasma which is partially stored in a marginal "still space." Hematocrit values of different organs and tissues of the dog¹³ having a venous hematocrit of 43 per cent were found to be as follows: spleen, 80; liver, 40; lungs, 35; heart and skeletal muscles, 20-25; and kidney, gastrointestinal tract and brain, 15-20. Another study in the dog¹¹ reported finding 31 and 49 per cent fewer cells in hepatic and renal blood, respectively, and 70 per cent more cells in splenic blood than in venous blood. Because of this unequal distribution of erythrocytes in large and small vessels, the venous hematocrit overestimates the body hematocrit or true ratio of cells to plasma in the vascular system as a whole. Investigations of this relationship have revealed mean ratios of body hematocrit to large vessel or venous hematocrit of 0.89 and 0.91 in the dog^{14,29} of 0.90 in man¹² and goat,⁹ and 0.92 in the sheep.³⁶ This correction factor for venous hematocrit has been designated the F_{cell} ratio and it may be used to correct the venous hematocrit when blood volume is calculated from the true plasma volume as determined by T-1824 or other plasma tagging methods.

$$\text{Blood volume in ml.} = \frac{\text{Plasma volume in ml.}}{100 - \text{venous hematocrit} \times F_{cell} \text{ ratio}} \times 100$$

There is some question regarding the use of a standard correction factor for F_{cells} in the dog or any other animal having a large active spleen.

The F_{cell} ratio in dogs has been reported to vary from 0.87 to 1.1 depending on the fraction of total erythrocytes in the spleen.¹⁵

For greatest accuracy in deriving a value for blood volume, the simultaneous direct measurement of red cell volume and plasma volume is required. Currently, Cr^{51} appears to be the most reliable and simple method for direct measurement of total red cell volume and either T-1824 or I^{131} labeled serum albumin is satisfactory for the plasma volume measurement.¹ An excellent summarization of the status of blood volume measurement up to 1959 is to be found in a review covering 292 literature references.³

Blood Volume in Animals. A few examples of reports from the literature on blood volume in domestic animals are presented in Table 1. The earlier reported values are subject to the errors inherent in the methods employed and only the few observations of recent years employing simultaneous cell volume and plasma volume determinations by direct measurement can be considered to be representative of the true total blood volume. The reports of Clark and Woodley²⁹ in dogs and of Stahl and Dahl³⁷ in growing calves verify the conclusions of others that blood volume calculated from T-1824 plasma volume measurement is significantly higher than the value obtained by calculation from the direct measurement of erythrocyte volume. Although blood volume derived from the simultaneous direct measurement of both plasma and red cells is regarded as providing a more valid result, in two separate studies^{29,34} there exists a difference of 13.4 ml./kg. of body weight between the reported mean blood volume in dogs. It appears that the true mean total blood volume in the dog awaits verification. For practical clinical application, the factor of 40 ml./pound of body weight is useful for predicting blood volume.

Blood volume studies in the cat, sheep, goat and horse are too limited for the establishment of firm values. Until additional data are forthcoming, a factor of 6.0 per cent of the body weight is suggested for predicting blood volume in the cat, sheep, goat, and "cold-blooded" horse. In the case of the "hot-blooded" equine, the work of Julian and colleagues³² suggests that a factor of 10 per cent of body weight should be considered.

Blood volume of the growing animal decreases as body size increases.^{28,37} In growing swine,²⁸ the red cell volume per unit of body weight decreased less than the plasma volume or total blood volume. In the swine of approximately 250 pounds body weight, the blood volume was about 25 ml./pound. This low value, as compared to blood volume in the dog, is the result of the large proportion of adipose tissue in swine; adipose tissue does not require the same volume of blood per unit of weight as lean muscle tissue.

The mature non-lactating bovine also has a low blood volume per unit

of body weight as compared to the dog. However, it has been pointed out³⁵ that if the weight of the four stomachs and their contents, estimated at about 160 pounds, is deleted from the body weight, the average blood volume per unit of body weight would be increased significantly. For practical application, a factor of 30 ml./pound is suggested for predicting total blood volume in the mature bovine and 35 ml./pound for growing dairy calves and lactating cows.

BLOOD STUDIES IN RELATION TO WATER BALANCE

When the amount of water eliminated from the body exceeds water intake, the animal enters a state of dehydration. Blood volume becomes reduced due to water loss but the formed elements in the blood and many of the chemical constituents show an apparent increase; these apparent increases are the result of *hemoconcentration*. The data obtained from the analysis of a blood sample must be interpreted in the light of the state of water balance of the animal. If dehydration is severe, and allowances are not made for it, a false impression of the state of the blood will result.

An example of this situation is the case of a 9-year-old Cocker male with the history of not eating and showing signs of listlessness for about 1 week. The temperature was 103° F. and physical examination revealed tenderness of the abdominal area. Blood samples were taken on arrival, and again on the first, fourth and tenth days of hospitalization. The data relative to the red cell series were as follows (also, see Appendix, case 2):

	<i>Arrival</i>	<i>1st day</i>	<i>4th day</i>	<i>10th day</i>
Hemoglobin gm./100 ml.	13.6	9.0	9.8	9.0
Erythrocytes in millions/cmm.	7.25	5.5	6.14	4.83
Volume per cent erythrocytes (Venous hematocrit)	43.0	31.0	32.5	30.0

The fluctuations during this 10-day period were due primarily to changes in water balance. On arrival, the animal was in a well advanced state of dehydration. The blood had become concentrated to the point that a measurement of hemoglobin, erythrocyte number and volume per cent of erythrocyte gave results indicating the red cell series to be well within the normal range. Twenty-four hours later, a 25 per cent reduction had occurred in the hemoglobin concentration and erythrocyte mass without the occurrence of actual blood loss. The explanation lies in dilution of the blood by water intake, returning the blood to its normal volume. When this occurred, the true composition of the blood could

be seen and it was apparent that the disease from which the patient was suffering was complicated by the existence of anemia.

Warm blooded animals constantly eliminate water from the body via the respiratory tract and skin in the dissipation of body heat, and from the urinary tract in the elimination of urea, salts and other end products of metabolism. This water elimination takes place even though water intake has stopped and the animal is becoming progressively dehydrated. Thus a sick animal that refuses to eat and/or drink enters a state of anhydremia. Dehydration, which results in hemoconcentration and reduction in blood volume, is hastened when the illness is accompanied by an increase in body temperature, sweating, diuresis, vomiting and/or diarrhea.

Marriott⁴⁴ in an excellent review of the problem of anhydremia makes the following points:

1. Sudden withdrawal of fluid from the body results in immediate concentration of the blood. Later, water is given up from the reserve stores in muscle and skin and the blood returns to normal. This would be the situation that pertains during and following strenuous exercise.
2. Water loss from the blood leads to an increase in total solids and therefore in specific gravity. Serum proteins may increase 50 to 100 per cent. Both the erythrocyte count and hemoglobin concentration increase.
3. There is an elevation of total non-protein nitrogen and urea concentrations in the blood which may be explained in part by functional disturbance of the kidneys and in part by increased destruction of body protein.
4. As the blood plasma becomes concentrated through water loss the inorganic salts are excreted in the urine in such amounts as to maintain an approximately normal concentration of the plasma.
5. Protracted dehydration leads to adjustments in the circulatory system and the blood:
 - (a) Erythrocytes and plasma proteins become diminished through actual destruction bringing about a lowering of blood viscosity. This in turn reduces the work-load on the heart. Upon administration of fluids, abnormally low erythrocyte counts and plasma protein concentrations may occur as hemodilution takes place in restoration of blood volume.
 - (b) Peripheral vessels become constricted greatly reducing the flow to the exterior of body and through the extremities. These compensations tend to maintain circulation of the reduced blood volume through the more vital organs.
 - (c) Pulse becomes small and rapid as heart rate increases and blood pressure is maintained. Prolonged dehydration leads

to reduced blood pressure as viscosity of the blood is lowered by destruction of proteins and red cells.

6. Secretion of urine is greatly diminished and the urine that is produced is of high specific gravity. Traces of albumin and numerous casts are regularly present. These changes are the result of functional insufficiency and inability of the kidney to separate a normal urine from a concentrated blood.
7. Miscellaneous manifestations of anhydremia:
 - (a) As much as 10 to 25 per cent of the body weight may be lost
 - (b) The skin becomes dry, wrinkled and loses its elasticity
 - (c) Mucous membranes become dry and lusterless
 - (d) Salivary secretion ceases
 - (e) Tongue and lips are shrivelled
 - (f) Extremities are cold
 - (g) Signs of air hunger appear as acidosis develops

DeBoer¹⁰ conducted experiments in chronic and acute dehydration in 19 dogs. The dehydration resulted in gradual hemoconcentration but toward the end of a 10-day period a decrease of blood constituents to slightly subnormal levels was observed. This observation is in agreement with item 5 (a) above. The change in body weight was most marked during the first 2 days, when about 10 per cent of the total weight was lost. By the end of the period about 25 per cent of the original weight had been lost. When the dogs were given water and food again, recovery was rapid, regaining 10 per cent of their normal weight during the first and second days. This was followed by a period of fluctuation and a gradual return to normal weight in about 25 days.

Skin and muscles are the principle depots of water¹² and combined they contained three-fourths of the available water in normal rabbits. When puppies 27 to 55 days old were deprived of water for periods of from 2 to 7 days,¹³ the water loss per 100 grams of fat-free dry substance as compared to controls was as follows: skin, 43 per cent; muscles, 35 per cent; brain, 14 per cent; liver, 13 per cent; and, kidneys, 9 per cent. Under conditions of severe dehydration, the skin gives up a greater fraction of its water than other tissues.

Underhill and Kapsinow¹⁵ state that concentration of the blood exceeding 25 per cent of the normal is attended by grave symptoms and that a level maintained at 40 per cent or more is usually followed by death.

HEMORRHAGE AND BLOOD RESTORATION

The escape of blood from the body, as in hemorrhage, obviously will have the effect of reducing blood volume. Drastic reduction in blood volume interferes with circulation of the remaining blood and the animal dies from shock.

Wang *et al.*⁴⁷ using 30 dogs determined by means of dye T-1824 that their blood volumes ranged from 86 to 112 ml. with an average of 98 ml./kg. of body weight. Under ether anesthesia, blood was removed from the femoral artery at the rate of 32 to 46 ml./kg. of body weight during a 15- to 20-minute period. Wang found that the residual blood volume at 50 per cent survival was 59 ml./kg. of body weight whereas at 84 per cent survival the residual blood volume was 69 ml./kg. body weight. Walcott⁴⁸ concluded that 60 per cent of the normal blood volume is the irreducible minimum in the canine compatible with survival. With less than this amount in the circulation the animal goes into progressive shock which, if untreated, results in a fatal outcome within a few hours. Walcott pointed out that an important factor in determining survival in massive hemorrhage is the reserve capacity of the body to shift fluid from the tissues to the vascular bed. That these shifts occur rapidly was shown by Adolph, Gerbasi and Lepore.³⁹ They measured changes in blood concentration in dogs at intervals of a few minutes before, during and after severe hemorrhage. Dilution of the plasma always occurred as a result of movement of fluid from extravascular spaces into the circulating plasma. The mean rate of this dilution initially was about 0.25 ml./kg. of body weight/1 minute, and required about 22 minutes for completion. Another study⁴¹ reports that following severe hemorrhage in dogs, the most pronounced hemodilution occurred in the first hour, but continued at a decreased rate for 72 hours.

The data on blood loss obtained by experimental bleeding of dogs indicate that, in acute hemorrhage, death from shock may occur following the loss of 25 to 30 per cent of the blood; that only about 50 per cent survival can be anticipated following a 30 to 40 per cent reduction in blood volume; and, finally death is almost certain to occur when above 40 per cent loss is sustained in a short period of time, if proper therapy is not administered. The fall in plasma proteins is greatest during the first hour following severe hemorrhage.⁴¹ Replacement of albumin begins within hours but is still incomplete at the end of the first week. On the other hand, the globulin fraction continues to fall for the first 7 hours and then is rapidly restored over a period of 24 to 72 hours. In the case of more gradual bleeding, the animal's body may sustain as much as 50 per cent loss of the pre-existing blood before grave symptoms appear. This is by reason of the fact that the slow loss of blood by hemorrhage provides time for movement of tissue water into the circulation to maintain the total volume and in addition thirst which attends hemorrhage will lead to the intake of water, if available. Symptoms that begin to appear when 50 per cent loss of the original blood volume has occurred are referable to the reduction of hemoglobin through loss of red cells. Other than release of stored erythrocytes by the spleen in hemorrhage as an emergency measure, the body is incapable of

replacing erythrocytes except through a process of maturation in the bone marrow which requires days rather than hours to be accomplished.

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Chapter 2

Materials and Methods for the Study of the Blood

THE discipline of hematology has much to offer the field of clinical veterinary medicine. The veterinary practitioner should select a few technics to serve as screening procedures on blood, the results of which, when combined with history and clinical examination, would offer valuable diagnostic information and also serve to indicate when additional and more specific procedures should be applied.

Much valuable information can be obtained from the mere inspection of an adequately centrifuged sample of blood. Such gross examination is readily accomplished by making use of the procedure commonly called the hematocrit. Wintrobe³¹ has made the following statement: "My own experience has convinced me that of the three instruments, the hemocytometer, the hemoglobinometer, and the hematocrit, the latter affords more information than the other two and the information is more accurate. . . . In experienced hands, the routine use of the hematocrit can indicate along what lines further study of the blood should proceed. This may well be the first blood study in those diagnostic clinics in which the technical personnel is too limited to permit routine performance of erythrocyte, leukocyte, and platelet counts as well as hemoglobin and Van den Bergh tests." With the hematocrit it is possible to determine the existence of anemia, hemoconcentration, extremes in leukopenia or leukocytosis, and occurrence of icterus.

The microscopic examination of blood, whether by stained film or wet mount, offers a great deal of valuable information relative to the response of the hemopoietic centers to disease. Knowledge relative to the morphology of the erythrocytes is essential to the understanding of the nature of an anemic process. The differential distribution of leukocytes will be helpful in both diagnosis and prognosis.

The routine use of the hematocrit and stained blood film in combination will reveal unsuspected derangements and in the many instances where the blood is found to be normal, such knowledge is also of value to the clinician. It should be realized that a specific diagnosis seldom can be made from laboratory findings alone but the laboratory results often provide clues which together with the history and physical examination make for a more critical diagnosis and provide a basis for prognosis and proper treatment.

THE MICROSCOPE

A good microscope, properly cared for, will last a lifetime. When purchasing a microscope one should give serious consideration to the quality of the lenses, the magnification and the advantages of a binocular over the less expensive monocular.

The Lenses. The *ocular* is the eyepiece lens and the *objective* is the lens in contact with the specimen. The cost of the microscope is influenced by the type of lenses in the objectives. *Achromatic* objectives are cheapest and for routine laboratory use they are suitable for low power and high dry objectives. However, high power oculars will give some color with achromatic objectives and especially when oblique light is used. In *fluorite* objectives some of the glass elements are replaced with natural mineral fluorite in order to partially correct for the occurrence of color or secondary spectrum. The most expensive objective is designated *apochromatic*. It consists of several fluorite lenses and additional correcting elements to remove the secondary spectrum and a correction is made for spherical aberration. It is advisable to request *coating* of the objectives with a thin film of low refractive index to increase light transmission and to reduce the intensity of reflected light from glass surfaces.

The *resolving power* of an objective refers to the ability of the lens to show distinctly separate elements only a short distance apart. The *numerical aperture* is a statement of the resolving power of the lens. The numerical aperture multiplied by 100,000 indicates the number of lines to the inch that can be resolved by the objective, *e.g.*, N.A. 0.25 = 25,000 lines to the inch or N.A. 1.25 = 125,000 lines to the inch.

Magnification of the specimen is determined by a combination of ocular and objective. The magnification provided is engraved on each and the objective magnification multiplied by ocular magnification equals the final magnification, *e.g.*, $97 \times$ objective and a $10 \times$ ocular provide a final magnification of $970 \times$. The *depth of focus* refers to sharpness with which all elements of the specimen can be seen. It is a function of both the numerical aperture and the magnification and is inversely proportional to both. Thus, the depth of focus becomes reduced as N.A. and magnification are increased. For this reason, it is important not to employ a greater magnification than necessary. The ideal depth of focus for most specimens is approached when the magnification is in the range determined by multiplying the numerical aperture by the factor 500. With an oil immersion objective of $97 \times$ and N.A. 1.25, the ideal depth of focus is approached at a magnification of 625. A $10 \times$ ocular and a $97 \times$ objective give a magnification somewhat in excess of the ideal for *depth of focus*. Sharpness of specimen detail would be improved by

selecting a $7.5 \times$ ocular for use with the $97 \times$ objective but some magnification would be sacrificed.

The *oil immersion* objective requires a drop of special oil to be placed between the objective and the slide. The oil should not be left on the objective when the microscope is not in use. The objective should be raised about 1 cm. above the microscope stage and the revolving nose-piece turned so that the oil immersion lens points away from the in-use position. The oil is removed from the front lens by wiping with special lens paper. Never use other paper or cloth to remove the oil. It is not necessary to use xylol on the lens paper when the oil is removed at the end of each period of use of the microscope. The *ocular* lens may become coated with a grease-like film from the eyelashes. This film is removed by fogging the lens with breath and wiping with lens paper. The *condenser lens* and *substage mirror* should be wiped with lens paper when oil contacts the former and dust appears on the latter.

Obtaining Correct Illumination. The mirror below the stage is employed to reflect light through the specimen and into the lens system. There is a *plane* and a *concave* side to each mirror; the plane mirror should be used with microscopes equipped with a condenser. If the concave mirror is employed together with a condenser, the apex of the cone of light falls within the condenser, rather than at the level of the microscope slide, reducing the effectiveness of the light beam.

The *microscope lamp* should be fitted with an iris diaphragm and a blue filter. A definite routine should be followed to obtain critical illumination for the improper use of light reduces the effectiveness of the microscope. The following outline of steps to be taken will lead to better illumination than obtainable by haphazard use of the microscope lamp, mirror and substage iris diaphragm.

- (a) *To position the lamp:* (1) place the lamp about 10 inches from the microscope, (2) remove the blue filter, (3) close the iris diaphragm to its smallest opening, (4) place the mirror in a vertical position with the *plane surface* toward the light source, and (5) spot the light in the center of the mirror.
- (b) *To obtain parallel light rays:* (1) close the iris diaphragm of the substage, (2) loosen the retaining screw on the lamp housing, (3) focus an image of the lamp filament on the leaves of the condenser diaphragm (view image in mirror), and (4) tighten the screw to retain the position of the light bulb.
- (c) *To obtain the desired amount of light:* (1) with both substage and lamp iris diaphragms closed, view the microscopic field and center the light source by adjusting the mirror; (2) open the substage iris just enough to obtain the maximum light that can be provided. Do not open the iris beyond this point. (3) Open the lamp iris in the same manner stopping at the point where best

lighting and definition are obtained. With the oil immersion objective in position, the iris of the lamp will be opened less than half at the point of correct illumination.

CLEANING GLASSWARE

Clean glassware is essential in the study of blood and time will be saved by making certain the glassware is clean before conducting a given procedure.

Slides. 1. New slides should be placed in isopropyl or 95 per cent ethyl alcohol and dried with a clean, soft cloth.

2. Used slides should be separated into two groups. (*a*) those with stain or immersion oil on them; (*b*) others with unstained blood films, *etc.*

Group (*a*) should be placed in hot water containing a detergent and chlorine solution, then washed individually with a cloth and scouring powder. They should be thoroughly rinsed in tap water, rinsed twice in distilled water and dried.

Group (*b*) should be left in potassium dichromate sulfuric acid cleaning solution* for several hours, then washed with a cloth and soap. They should then be rinsed thoroughly in tap water, twice in distilled water, and dried. If the slides are handled carefully, they will be suitable for re-use for blood films.

Hematology Pipettes. A water-pump aspirator attached to the faucet is essential for efficient cleaning. The aspirator is used to draw distilled water through the pipette until clean followed by acetone sufficient to dry. When the pipettes are clean and dry, the glass bead in the bulb will move freely. Pipettes should be inspected frequently to see that scum is not adhering to the inside, especially in the capillary portions. If not absolutely clean, fill with dichromate cleaning solution and after overnight action, blow the acid out of the pipette by means of rubber tubing followed by cleaning of the pipette in the usual way.

Hematocrit Tubes. A stainless steel U-shaped tube called the hematocrit tube cleaner is connected to a water suction pump by means of a piece of rubber tubing about 2 feet long. The hematocrit tube is inverted on the metal tube and the two are placed into a beaker of water (Fig. 1). When the suction is turned on, the blood is withdrawn from the hematocrit tube and water follows. After all the water has been sucked through the hematocrit tube, the suction is continued for a short time to draw air into the tube to dry it. The hematocrit tube should be cleaned frequently

*Commercial potassium dichromate	60 gm.
Tap water	300 ml.
Heat to dissolve, cool and then add slowly with continuous stirring commercial sulfuric acid	460 ml.

with dichromate cleaning solution by filling the tube with the solution and leaving it act overnight or longer or by filling the tube with strong chlorine solution and cleaning after about 15 minutes.

Hemocytometers. Hemocytometers and cover glasses should be rinsed with distilled water and dried with clean facial tissue or a soft clean cloth after each use. Polish with clean facial tissue before each use for the counting chamber will not fill properly if not absolutely clean. Care must be taken not to scratch the chamber or cover glass.



FIG. 1. A convenient method for cleaning the Wintrobe hematocrit tube by means of a water vacuum pump.

General. Miscellaneous glassware, such as syringes, flasks, beakers, and pipettes, should be washed in hot water with detergent, rinsed thoroughly with tap water, 2 or 3 times with distilled water, and allowed to dry. In cases where this does not get glassware clean, use cleaning solution overnight followed by cleaning as described above.

COLLECTING AND HANDLING BLOOD FOR LABORATORY STUDY

In veterinary medicine, the usual procedure with large domestic animals is to collect 5 ml. of blood directly into a shell vial containing

the proper amount of anticoagulant in dry form. However, with the canine, the blood is drawn by means of a dry glass syringe, and transferred to the shell vial after removing the needle. A 5 ml. volume of blood is adequate for routine blood study but in the case of the smaller breeds of dogs it is advisable to limit the volume to 2 ml. especially when some difficulty is anticipated. However, it will be necessary to have available a shell vial with the indicated smaller quantity of anticoagulant.

The cork used to close the vial should be coated with paraffin so that when the blood is mixed prior to removal of portions for various tests, the blood will not leak from the vial or stick to the cork. If rubber stoppers are used, the paraffin coating is not required.

Anticoagulants. The *oxalates* are the most commonly employed anticoagulants for routine blood study. They are poisonous and for this reason they should never be used where blood is collected for purposes of transfusion. Sodium citrate is employed for the latter purpose. The oxalates and sodium citrate prevent clotting by interference with the calcium ion.

Heller and Paul² determined that about 0.2 per cent final concentration of oxalate or 0.4 per cent citrate produces the most favorable result from the standpoint of anticoagulation and hemolysis. The inorganic salts have the effect of altering the volume of the erythrocyte³ so that cell-plasma ratio varies with the type and concentration of anticoagulants. Ammonium salts increase cell volume whereas sodium, potassium, and lithium salts produce an opposite effect. Heller and Paul proposed a combination of the potassium and ammonium oxalates to be prepared and employed as follows: 0.8 gram potassium oxalate (shrinks cells) and 1.2 grams ammonium oxalate (swells cells) are dissolved in 100 ml. of distilled water and dispensed into shell vials at the rate of 0.1 ml. for each 1.0 ml. of blood to be drawn. The anticoagulant is evaporated to dryness in an oven at 60° C. This anticoagulant formula has the advantage of being inexpensive and easy to prepare and use. However, it is necessary to initiate the examination of the blood specimen within the first hour insofar as erythrocyte sedimentation rate and preparation of films for the leukocyte differential count are concerned for in oxalated blood ESR becomes decreased and leukocyte disintegration occurs with aging of the sample. When blood is drawn for non-protein nitrogen determination, the double oxalate is not suitable by reason of the ammonium salt. Lithium oxalate is used at a concentration of 1.0 mg./ml. of blood for non-protein nitrogen determination.

Heparin is a natural anticoagulant occurring in various tissues and it is found abundantly in the liver from which its name is derived. Heparin prevents blood coagulation by interfering with the conversion of prothrombin to thrombin. It does not alter erythrocyte volume and, there-

fore, it is suitable for the hematocrit determination⁶, but it has a serious disadvantage in that stainability of leukocytes in heparinized blood is inferior to stainability of leukocytes in oxalated blood. Heparin is commonly employed as a 1.0 per cent solution at which concentration 0.1 ml. is adequate to prevent coagulation of 5.0 ml. of blood. A syringe may be rinsed with stock heparin solution and sufficient anticoagulant is retained to prevent coagulation as blood is drawn into the syringe. Another method of use described by Wintrobe⁷ is to dissolve heparin in water in a concentration of 7.5 mg./1.0 ml. This amount is placed into the shell vial and allowed to dry at room temperature. A thin film of heparin will form along the sides and bottom of the vial and this will enhance rapid mixing of the heparin with the blood. This amount of heparin will prevent the coagulation of 5 ml. of blood. Heparin is about 150 times more expensive than the oxalates.

The *disodium salt of ethylenediaminetetraacetic acid*, also known as EDTA, Sequestrene, disodium versenate, or versene, was introduced as an anticoagulant in 1942 by Dyckerhoff, Marx and Ludwig of Germany. The first published report of its use in America as an anticoagulant in routine blood study was by Proescher⁵ in 1951. The disodium salt has a maximum solubility in water of 10 per cent and 0.005 to 0.01 ml. (0.5 to 1.0 mg.) is sufficient to prevent coagulation of 5.0 ml. of blood. Several reports on the new anticoagulant ascribed to it a number of characteristics that reveal it to be the ideal anticoagulant and blood preservative for use in routine blood examination. The dipotassium salt is also suitable and it has the advantage of being more soluble than the disodium salt,¹ but it is much more costly.

EDTA does not alter erythrocyte size. Erythrocyte sedimentation rate does not change during the first 6 hours with the blood sample at room temperature and may be unchanged for up to 24 hours in samples held in the refrigerator.⁴ Leukocyte stainability is excellent and the integrity of the cells is preserved for 6 hours or longer. Results of a comparative study by Schmidt, Hane and Gomez⁶ of the effect of potassium oxalate, heparin, and EDTA on blood smear morphology and stainability are summarized in Table 2.

In addition to the excellent results with EDTA on blood morphology it was reported that EDTA-blood was in agreement with heparinized or oxalated bloods in determinations for creatinine, urea nitrogen, glucose, phosphorus and uric acid. However, chloride determinations in EDTA-blood were always higher than results with the other anticoagulants.⁶

EDTA is a powerful anticoagulant which binds the blood calcium ion. It was stated by Proescher⁵ that 0.5 to 1.0 mg. is adequate to prevent coagulation of 5 ml. of blood. Schmidt *et al.*⁶ employed 2.0, 3.0 or 10.0 mg./5 ml. of blood and concluded that no differences in cell morphology or stainability resulted from the three concentrations em-

ployed. Melville and Rifkind⁴ used 1.0 mg./ml. of blood in their studies on erythrocyte sedimentation rate. Wittgenstein⁸ employed 0.05 to 0.1 ml. of a 10 per cent solution for 5 ml. of blood which corresponds to a concentration of 1 to 2 mg./ml. of blood. It appears from these reports that the concentration of EDTA may be varied from 0.5 to 2.0 mg./ml. of blood without significant adverse affect on the laboratory results obtained.

Table 2. The Effect of Three Anticoagulants on Blood Smear Morphology

<i>Observation</i>	<i>Time Interval (hours)</i>	<i>Potassium Oxalate (Heller & Paul Formula)</i>	<i>Heparin (0.1 ml. of 1.0% / 5 ml. of blood)</i>	<i>EDTA (2.0, 3.0 or 10.0 mg./5 ml of blood)</i>
Autolysis of cell nuclei . . .	1	+	++*	—
	6	++	++	+
	24	+++	++	++
Clarity of cell morphology . . .	1	+++	++	++++
	6	++	+	++++
	24	+	+	++++
Stainability	1	++++	++	++++
	6	+++	++	++++
	24	+++	++	++++
Quantity of platelets . . .	1	++++	++	++++
	6	++	+	++++
	24	+	+	++++

* Cells distorted and poorly preserved.

** Cells very hazy.

Early in the year of 1960, disodium versenate replaced the double oxalate formula of Heller and Paul² as the anticoagulant for blood drawn from all animal patients in the veterinary clinic of the University of California. A concentration of approximately 2.0 mg./ml. of blood was found to be satisfactory and this was dispensed as the dry powder into the shell vial. A smooth, shallow pit of sufficient volume to trap 10.0 mg. of disodium versenate was cut into the surface of a wooden tongue depressor. This special spatula is dipped into the dry disodium versenate and then is drawn across the edge of a second tongue depressor to fill the pit and remove excess powder (Fig. 2). The quantity of disodium versenate retained on the spatula is transferred to a shell vial for use in the collection of 5 ml. of blood. This method saves time as compared to dispensing a liquid anticoagulant into vials and then evaporating to dryness. In fact, difficulty with solubility of the disodium salt in blood is experienced when it is first dissolved in water and then evaporated to dryness as is customary with the double oxalate formula. Results with disodium versenate have been entirely satisfactory from the standpoint

of the routine laboratory determinations. The stained blood films have been superior to films previously obtained from oxalated blood samples. The fact that the blood is well preserved up to 6 hours at room temperature by EDTA has been especially advantageous for blood samples collected from animals on farms located some distance from the laboratory.

The data on blood values in normal and diseased animals originating at the University of California, School of Veterinary Medicine and in-



FIG. 2. Method for dispensing EDTA anticoagulant powder into vials. The pit in the end of the spatula containing the powder holds 10 mg. EDTA when filled level with the surface of the spatula. The pit in the opposite end of the spatula is for dispensing 4 mg. of EDTA anticoagulant powder.

cluded in this first edition of *Veterinary Hematology* were obtained from oxalated blood samples (Heller and Paul formula²) collected before the introduction of disodium EDTA as the anticoagulant for routine blood examination.

Obtaining the Blood Sample. In the case of large animals, such as the sheep, cow, and horse, from which a free flow of blood can be obtained from the jugular vein, the needle of proper size is introduced first through the skin and then into the distended vein and the blood is collected to the desired level directly into the vial containing the anticoagulant. In drawing blood from small animals (dog, cat), or when heart puncture is employed as with swine, the blood is collected in a clean dry, ground glass syringe. To prevent hemolysis from occurring, certain precautions must be taken. The needle and syringe must be dry for

hypotonic moisture will rupture some red cells; the blood should flow freely into the syringe with minimum use of suction or pumping action on the plunger; and, before transferring the blood from the syringe to the vial, the needle should be removed, for forcing the blood through the needle under pressure may rupture red cells. The syringe should be taken apart and rinsed, immediately, in order to prevent sticking of the plunger to the barrel.

Blood is commonly obtained from the various animals as follows:

<i>Animal</i>	<i>Site</i>	<i>Needle</i>	<i>Size</i>
		<i>Gauge</i>	<i>Length in Inches</i>
Horse . . .	Jugular vein	12-14	2½-3
Cow . . .	Jugular vein	12-14	2½-3
Sheep & Goat .	Jugular vein	14	2½-3
Pig . . .	Anterior vena cava	20	1½-4
Dog . . .	Cephalic, saphenous or jugular vein .	20-22	1½
Cat . . .	Cephalic or jugular vein	22-25	1
Rabbit . . .	Cardiac puncture	18	3

Stubbs¹⁴ has described the repeated bleeding of sheep for the purpose of obtaining rather large volumes of blood for laboratory use. The bleeding is done from the jugular vein using a 12-gauge, 3-inch needle. Lindenstruth and Ward¹³ have employed intracardial puncture in sheep for obtaining either small or large amounts of blood, for which purpose a 15-gauge, 3-inch needle is employed. Carle and Dewhirst¹⁰ were the first to employ the anterior vena cava as the site for bleeding of swine (Fig. 3). Hoerlein, Hubbard and Getty¹¹ highly recommend this method for bleeding of swine and they have described restraint, equipment, and special anatomical considerations for use of the method. Prior to discovery of this method, swine were bled by snipping the end of the tail or nicking a vessel in the ear. Methods have been described for bleeding small animals such as mink and ferrets,⁹ guinea pigs¹⁵ and mice.¹² In cats, blood is obtained by pricking an ear vein and pipettes are filled directly from the blood as it escapes from the punctured vessel.

Handling of the Blood. The vial should be tightly stoppered and tipped back and forth a dozen times to dissolve the anticoagulant. Mixing of the blood should always be done with gentleness, for rough handling may lead to rupture of red cells.

When the blood is allowed to stand in the vial, separation of cells and plasma takes place. The rate of settling of cells varies with animal species and disease. Horse blood often separates within minutes; dog, cat and pig blood may be rapid or slow in separation; cow, sheep and goat bloods seldom settle-out. Because of the tendency of the cells to settle, it is necessary to mix the sample thoroughly each time a portion is removed from the vial for a test. In a laboratory where many blood

samples are handled, it is convenient to employ a vertical turn-table to which the vials are attached by clips for purpose of obtaining a uniform suspension of the blood cells in the plasma (Fig. 4).

When *oxalates* are employed for anticoagulation, the blood studies should be commenced immediately; if there is to be a delay of an hour or longer, the vial should be placed in the refrigerator. However, the blood film should be prepared immediately for changes in morphology of leukocytes due to aging in the presence of oxalate may begin to appear

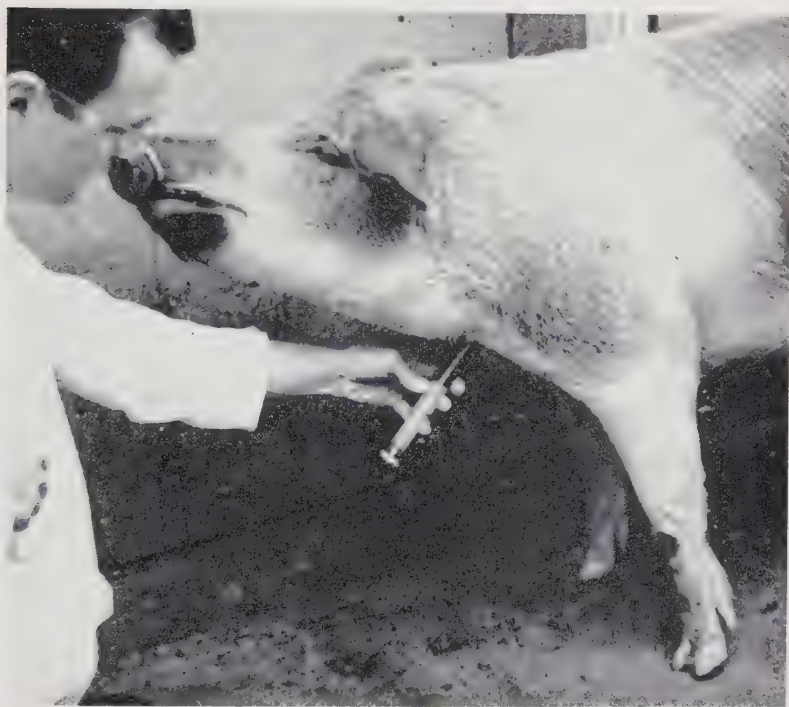


FIG. 3. Collecting blood from the anterior vena cava of a 200-pound pig.

within 20 to 30 minutes, and these changes become more advanced as the age of the sample increases. The cells of the erythrocytic series are much more resistant to the influences of aging in respect to their morphology but their *sedimentation rate* changes as the sample ages; the change is usually one of retardation in rate of settling. Total erythrocyte and leukocyte counts and hemoglobin determination may be delayed as long as 24 hours, without introducing serious errors, providing the blood is kept cold. The Wintrobe hematocrit test probably could be conducted up to 24 hours after collection without seriously affecting the volume per cent of erythrocytes. These difficulties are obviated

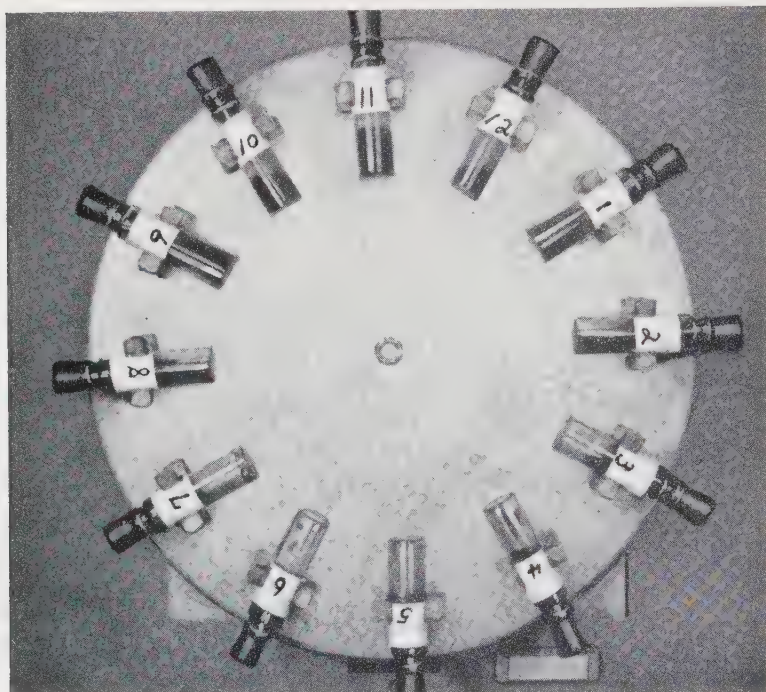


FIG. 4. A vertical turn-table for thorough mixing of the blood sample when laboratory studies are in progress.

when EDTA is substituted for oxalate as the anticoagulant. However, in routine blood studies a definite procedure should be followed and all tests should be completed within a reasonable time period. The following sequence of tests is suggested to the student of hematology:

1. Prepare the blood film and set it aside in a protected place until it can be stained.
2. Fill the hematocrit tube for the erythrocyte sedimentation test and/or hematocrit determination.
3. Stain the blood film.
4. Determine the hemoglobin concentration in grams per cent.
5. Make the erythrocyte and leukocyte counts.
6. Study of the stained blood film for red cell morphology, presence of thrombocytes, and for the differential distribution of the leukocytes.

THE BLOOD FILM

The blood film should be made as soon as possible. It may be prepared on a slide or on a cover-slip. The slide method is perhaps more applic-

able to veterinary medicine as practiced on large animals where blood films may be prepared at the farm, although, either the slide or cover-slip method is suitable for use in the laboratory of the animal hospital.

Slide Method. A clean slide is placed on a level surface and a very small drop of thoroughly mixed blood is deposited near one end of the slide. The inexperienced person has a tendency to use too large a drop of blood. A round applicator stick may be used to transfer blood from the vial to the slide and to control the size of the drop. Spread the blood in an even film by means of another slide. The spreading edge should be perfectly smooth (free of chipped spots) and the corners of the slide should be cut off in order to make the spreading-edge slightly shorter

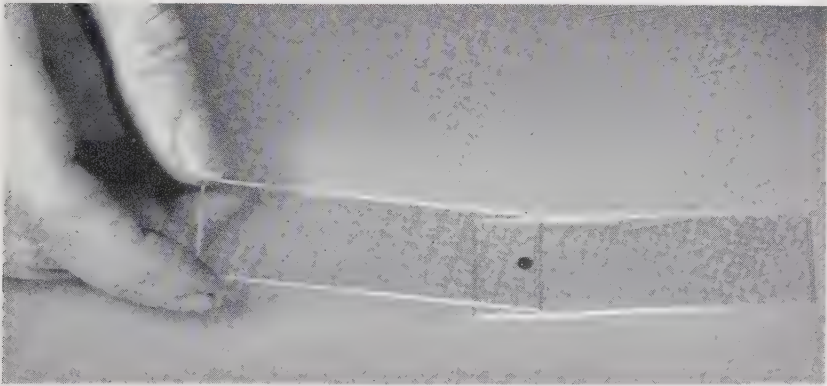


FIG. 5. Method for preparing a blood film on a slide. (1) The spreader slide is drawn toward the small drop of blood; (2) as soon as contact with the blood is made, the spreader is moved forward in a smooth glide.

than the width of the slide on which the film is to be made. The spreader slide is laid on the inner surface of the index finger or held between the thumb and finger, and drawn toward the drop of blood (Fig. 5); as it makes contact, the blood will spread along the edge of the spreader slide which is then moved forward in a smooth glide. The angle at which the spreader slide is held determines the thickness of the blood film, *i.e.*, the greater the angle, the thicker the film. Generally, about a 30 degree angle is satisfactory. The slide is waved in the air to hasten drying, for slow drying leads to movement of water out of the erythrocytes into the plasma resulting in *crenation* of the erythrocytes. Thin films and fast drying are essentials for perfect blood films. The criteria of a good slide blood film are:

- (a) *Smooth, even appearance, and free from holes.* A wavy film is due to a jerking movement when spreading and holes in the film are usually caused by grease or dirt on the surface of the slide. Clear streaks are the result of a chipped edge of the spreader slide.

- (b) *The film has long, straight borders that are about 2 mm. in from the edge of the slide.*
- (c) *The erythrocytes are distributed in a single layer over a greater portion of the film. Films that are too thick are difficult to study. The leukocytes must have space in which to spread so that cytoplasmic detail can be seen.*



FIG. 6. An applicator stick is used to place a small quantity of blood on a cover-slip glass in preparation for making a blood film.



FIG. 7. In preparing blood films on cover-slips, a second glass is dropped cross-wise onto the cover-slip containing the small drop of blood. The blood spreads quickly between the glasses and at the moment that spreading is complete, the two glasses are separated by sliding them apart.

- Cover-slip Method.* 1. Holding the cover-slip by its edges in one hand, place a small drop of blood (1-2 mm. in diameter) on its surface by means of an applicator stick (Fig. 6).
2. Quickly drop another cover-slip on the first in crosswise fashion. If the cover-slip is clean, the blood will spread quickly and evenly between the two surfaces (Fig. 7).
 3. Just before spreading is complete, separate the two cover-slips by sliding (not lifting) them apart in a horizontal manner.
 4. Wave the film in the air to dry it.
- Care of the Blood Film.* 1. Stain the film immediately, if possible, otherwise store it in a protected place and stain within 24 hours for best results.
2. Blood films made at the farm should be protected for flies may devour erythrocytes, and within minutes the film may be full of holes.

BASIC PRINCIPLES FOR STAINING BLOOD

An understanding of the physical-chemical principles involved in the differential staining of blood cells is prerequisite to the satisfactory use of Wright's or Giemsa's stains. Romanovsky (1891) was the first worker to combine eosin and methylene blue in the staining of blood. He realized that the differential staining effect obtained was not entirely due to the two stains but additional dyes had formed. The present day blood stains which employ eosin and methylene blue are commonly referred to as "modified Romanovsky stains."

Methylene blue readily oxidizes to form azure dyes called Azure A, B, and C. Methylene blue solution, upon standing or aging, forms the azure dyes and is then referred to as "polychrome methylene blue." Oxidation of methylene blue can be hastened by boiling in the presence of alkali. Wright's stain is methylene blue polychromated with sodium bicarbonate and heat to which eosin is added. Eosin enters into chemical combination with the basic dyes present and a compound insoluble in water precipitates. This precipitate is the Wright's stain powder which is soluble in absolute alcohol.

Giemsa stain consists of Azure II-eosin and Azure II in the ratio of 15:4. Azure I and II are trade names and it is said that Azure B predominates in Azure I and that Azure II is a mixture of Azure I and methylene blue in equal parts.

The azures are basic dyes and therefore they are attracted to the acid nuclei (nucleic acid), whereas the eosin is an acid dye and it is attracted to the alkaline cytoplasm. This combination of basic and acid dyes, in the form of chlorides for the basic dyes and sodium or potassium salts of the acid dyes, is referred to as a "neutral stain."¹⁸

The "neutral stains" are soluble in absolute methyl or ethyl alcohol, but the alcoholic solutions tend to retain the dye so that it is not adsorbed to the protoplasm of the cell. Thus, it is necessary to force the dyes out of solution by the addition of water which precipitates the stain. The water must be of proper pH in order to provide the best differential staining. Water that is too alkaline favors the methylene blue and azure stains, causing the cells to become too blue; whereas too acid a medium for staining leads to over-emphasis of the eosin stain with little or no staining on the part of the nuclei of cells.

Blood and tissue cells show a greater affinity for the stain after treatment with a mordant. Blood cells are prepared for staining by fixing with absolute methyl alcohol. The methyl alcohol should be neutral in reaction and free of acetic acid. Previously, it had been recommended that the alcohol be acetone-free, but Conn¹⁸ states that this is not important. The same purity of methyl alcohol is required for use in dissolving the powdered stain in making the stock solution.

Wright's stain is used in concentrated form, while Giemsa's is employed as a diluted stain. Thus, staining with Wright's method is accomplished more rapidly but may lead to more difficulty. The fixation of the blood film in Wright's method is accomplished by first flooding the concentrated stock stain on the film and after *1 minute of fixation an equal number of drops of buffer or neutral water are added*. The diluted stain is allowed to act for about 4 minutes (staining period) after which the slide is briefly washed in tap water and dried. Buffer of pH 6.6-6.8 is used when neutral water is not available. Distilled water is usually too acid due to dissolved CO₂ and tap water may be too alkaline.

With Giemsa's stain the blood film is fixed in absolute methyl alcohol before exposure to the diluted stain. The Giemsa stain is diluted in water to a much greater extent than is the Wright's; therefore with Giemsa's, the staining of the cells takes place much more slowly requiring 30 to 45 minutes. This prolonged staining period is often a disadvantage but is off-set by the fact that more uniform results are obtainable with Giemsa's stain.

MATERIALS AND METHODS USED IN STAINING THE BLOOD FILM

Neutralization of Distilled Water. Gradwohl¹⁹ suggests using a few crystals of hematoxylin as a rapid means for testing the distilled water for neutrality. Rinse a clean test tube in the water to be neutralized and to 5 ml. of the water add a few crystals of hematoxylin.

Neutral water —becomes pale lavender in 10 seconds

Acid water —becomes yellow and remains yellow for more than
5 minutes

Alkaline water—becomes reddish purple immediately or within 1 minute

Neutral water may be prepared by adding 1 per cent potassium carbonate, drop by drop, to acid water in quantity sufficient to neutralize it or by adding 1 per cent HCl to alkaline water. The neutralized water should be prepared each day and it should be kept in a pyrex glass bottle with no rubber connections.

Phosphate buffer, pH 6.6, for use with Wright's stain in lieu of neutral distilled water is prepared as follows:

3.80 grams Na_2HPO_4

5.47 grams KH_2PO_4

Dissolve in 500 ml. distilled water in a liter flask and add distilled water q.s. 1000 ml.

Standard Wright's Stain. 0.1 gm. of powdered stain is placed in a mortar; 60 ml. of absolute methyl alcohol (acetic acid-free) is added a few ml. at a time while the powdered stain is ground to dissolve it in the alcohol. Continue grinding for about 5 minutes after all alcohol has been added. Transfer to a tightly stoppered brown bottle and store in the dark. Allow the stain to age for 2-4 weeks and filter it through paper before using. Aging may be hastened by storing at 37°C .

*Modified Wright's Stain.*²¹ 300 mg. powdered Wright's stain and 30 mg. powdered Giemsa's stain are ground in a mortar until dissolved in 100 ml. of absolute methyl alcohol. Let stand for 24 hours and use in the same way as the standard formula for Wright's stain.

Staining Blood Films on Slides by Wright's Method.

1. To conserve stain, mark-off with a China pencil the area of the film to be stained.
2. Place the slide on the staining rack and with a medicine dropper add 4 to 10 drops of stain. *Allow the concentrated stain to act 1 minute.* (Fixation period—sufficient stain must be added so that the alcohol will not evaporate during this period.)
3. Add an equal number of drops of buffer or neutral water and mix thoroughly by blowing or by applying air from the medicine dropper by pumping the bulb. Mixing is continued until a metallic film forms on the surface of the stain-buffer mixture.
4. Let the diluted stain act for from 2 to 4 minutes (staining period). Each batch of stain has its own time requirements. When testing a new batch of stain it is suggested that a *4-minute period* be used and to be followed by time modification as needed. With Modified Wright's formula, a staining time of 2-3 minutes has been satisfactory.
5. Float-off the scum and precipitated stain with neutral water or tap water. This is a most important step. It is accomplished by

quickly flooding water from a 50 ml. beaker over the slide.
EXCESSIVE WASHING WILL WASH-OUT THE STAIN.

Modifications for Staining Slides With Wright's Stain.

1. Fix the blood film in absolute methyl alcohol for 30 seconds and remove the surplus alcohol.
2. Cover the film with diluted Wright's stain which should be freshly prepared by thoroughly mixing 4 ml. stock stain with 6 ml. of distilled water or buffer.
3. Allow the diluted stain to act for 4 minutes or longer, depending on strength and age of the stain.
4. Rinse the film with water and air dry.

Staining Blood Films on Slides in Coplin Jars. Maintain Wright's stain and buffer in separate coplin jars fitted with screw lids. A few drops of stain should be added to the buffer.

1. Place the slide into the jar of Wright's stain.
2. After 2-5 minutes, using a forceps, transfer the slide directly to the coplin jar containing buffer.
3. After 2-5 minutes, remove the slide from the buffer and *rinse it briefly* in tap water and then air dry.

The advantage of the coplin jar method over the tray method is that less trouble is encountered with precipitate on the stained film.

Directions for Staining Blood Contained on Cover-slips.

A. Tray method:

- (1) Set the cover-slip on a cork which is standing on the bottom of the staining tray. The diameter of the cork should be smaller than that of the cover-glass.
- (2) With a dropper, cover the glass with stain, counting the number of drops needed.
- (3) After 2 minutes add an equal number of drops of buffer.
- (4) After 2 to 4 minutes, wash under a stream of tap water from a beaker being careful not to over-wash. Stand the cover-slip on end to dry in the air.
- (5) When thoroughly dry, mount the cover-slip by putting a small drop of piccolyte (or Canada Balsam) on a slide and place the cover-slip, film down, on the piccolyte, allowing it to spread under the entire cover glass. It can now be examined under the high dry objective or with the oil immersion lens.

When removing immersion oil from a cover-slip, wipe it off with a gentle motion or the cover-slip will be loosened. It is a good idea to let the piccolyte harden for 48 hours before attempting to remove the immersion oil from the cover-glass.

B. Coplin jar method:

Special small jars designed for cover-glasses are available. The jar should contain enough stain to cover the cover-slips. The buffer can be used for about 12 cover-slips before it need be replaced.

- (1) Place the cover-slip in the coplin jar containing Wright's stain. Keep the jar covered at all times.
- (2) After 2 minutes, using a forceps, transfer the cover-slip to the coplin jar containing buffer.
- (3) After 2 minutes in buffer, wash the cover-slip in tap water but do not over-wash. Dry and mount on a slide as described above.

Wright's-Leishman Stain. This method of staining blood films was substituted for the modified Wright's stain (Wright's-Giemsa) at the beginning of 1960 in the Clinical Pathology Department, School of Veterinary Medicine, University of California. About the same time the double oxalate anticoagulant (Heller and Paul formula) was replaced by EDTA. After these changes were made, it was observed that basophil leukocytes were being observed in canine blood (Chapter 3, Plate III) whereas previously this cell was seldom recorded as being present. It is probable that the methods employed prior to the beginning of 1960 failed to stain the basophilic granules and that in the absence of such staining the cells were designated as monocytes. The Wright's-Leishman stain is prepared as follows:

1. *Solution I, Wright's Stain.* Place 0.5 gram of powdered Wright's stain, 5.0 ml. of glycerin, and 300 ml. of absolute methyl alcohol into a 500 ml. pyrex flask. With extreme caution and with constant agitation, gently heat the mixture until the solution reaches a temperature that is just below the boiling point. Permit the solution to cool a few minutes and repeat the heating process 3 or 4 times. When the solution cools to room temperature, it is filtered and it is then ready to use.
2. *Solution II, Leishman Stain.* Substitute 0.5 gram of powdered Leishman stain in the above formula and prepare the solution for use in the same manner.
3. *Wright's-Leishman Stain.* The final staining solution is prepared by mixing 3 parts of solution I and 1 part of solution II. The mixture is stored and used in the same manner as Wright's stain or the modified Wright's stain with the exception that staining time (interval of exposure to buffer) should be increased to 6 minutes. When coplin jars are employed, the buffer should be fortified with stain in the proportion of 1 part stain to 5 parts buffer.

Giemsa's Stain.

NOTE. Commercial Giemsa's stain, liquid, is used for either slides or cover-glasses. The coplin jar method is preferable to prevent evaporation during the 30-minute staining.

1. Place the slide or cover-glass containing the blood film in methyl alcohol for 3-5 minutes and air dry.
2. Fill a coplin jar with Giemsa's stain which is made as follows:
To each ml. of neutral distilled water, add 1 drop of liquid Giemsa's stain. The large coplin jar holds about 60 ml., therefore, to 60 ml. of water add 60 drops of stain. The small coplin jar holds 9 ml. of water and 9 drops of stain.
3. Put the slide into the coplin jar of stain and leave it exposed to the stain for 30 minutes.
4. Wash the slide with neutral distilled water for 30 seconds and allow it to dry in the air. If cover-glasses are used, mount the film on a slide as described above.

Staining and Counting Reticulocytes.

The stain consists of 1 per cent brilliant cresyl blue in 0.85 per cent saline.

1. With an eye dropper, put an equal number of drops of blood and of stain into an empty vial.
2. After 5 minutes make a slide film or a cover-slip film from the mixture.
3. Counterstain with Wright's stain as described for a blood film.
4. Count 1,000 erythrocytes under high magnification and note the percentage of reticulocytes. A method for reducing the time involved in counting red cells through use of the Miller ocular disc has been described.¹⁷

Causes and/or Correction of Poor Staining.

- A. *Irregularity in staining intensity of various regions of the film:*
 - (1) Failure to obtain thorough mixing of stain and buffer.
 - (2) Variation in pH of surface of slide due to careless cleaning. Traces of either acid or alkali on the slide will ruin the film.
 - (3) Water allowed to stand on parts of film during drying. Always stand the slide or cover-slip on end to hasten drying after washing.
- B. *Washed-out appearance of the stain of entire film:*
 - (1) Water was allowed to act too long in washing the slide or the slide was not drained dry quickly enough.
 - (2) Insufficient staining time or failure to add a sufficient number of drops of buffer to the stain after the fixation period.
- C. *Nuclei stain faintly but erythrocytes and granules of eosinophils take intense eosin stain:*

- (1) The water used to dilute the stain or the buffer was too acid.
 - (2) The film was washed with acid water.
 - (3) Surface of slide was acid.
- D. *Nuclei intensely blue, erythrocytes greenish or blue, cytoplasm of leukocytes not stained:*
- (1) Water or buffer used in staining or washing was alkaline.
 - (2) Surface of slide was alkaline.
- E. *Film covered with precipitate:*
- (1) Failure to float-off the stain from the slide.
 - (2) Not enough stain placed on the slide during the period of fixation so that the alcohol evaporated leaving a precipitated stain.
- F. *Over-staining:*
- (1) Remove stain by dipping the film in 95 per cent ethyl alcohol and restain reducing the staining time.

MICROSCOPIC EXAMINATION OF THE BLOOD FILM

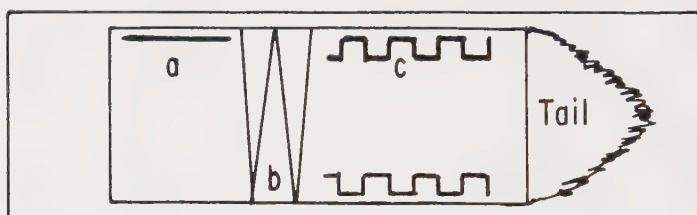
Initial study of the blood cells and their distribution is conducted with high dry magnification. Blood smears on slides without cover-slips must be coated with a thin film of immersion oil before satisfactory inspection is possible. Removal of oil is accomplished by wiping the slide with facial tissue or cheesecloth. Determine from high dry magnifications: (*a*) the degree of variation in size and shape of the erythrocytes; (*b*) existence of excessively high or low numbers of leukocytes; and, (*c*) the occurrence and relative distribution of thrombocytes.

The differential leukocyte count is preferably conducted with the oil immersion magnification, but with experience it may be accomplished under the high dry lens. The oil immersion lens, however, is necessary to see the azurophilic granules in monocytes, toxic granulation in neutrophils and certain blood parasites such as *Anaplasma*, *Theileria*, and *Haemobartonella*.

When blood is spread on a slide, there is a tendency for the neutrophils to rush to the edges of the film, the lymphocytes to remain mainly in the body of the smear, while the monocytes and eosinophils tend to become evenly distributed throughout. MacGregor, Richards and Loh²⁰ compared three methods for making the differential count in blood films on slides. The straight edge method which follows the edge of the film gave consistently higher neutrophil and lower lymphocyte counts than were obtained by the cross-sectional method which consists of going back and forth across the slide. These workers found the battlement method (Fig. 8) of counting to give values that compared favorably with the differential leukocyte count made by examination of the entire blood film. In describing the battlement method, they state: "It implies

a count made of three horizontal edge fields followed by two fields toward the center (so as to give three vertical fields), followed by two fields in a horizontal and then two fields in the vertical direction to reach the edge again." This limits the counting of cells in an area of 1 mm. of the edge of the blood film. In order to use the battlement method of counting, it is necessary to obtain films having reasonably long straight edges.

MacGregor *et al.* further state that in cover-slip blood films the cells tend to gather toward the center and provided a large enough number of cells are counted in the central area a good average figure is obtained.



a = Straight-edge method
b = Cross-sectional method
c = Battlement method

FIG. 8. Three methods for selecting areas for the differential leukocyte count in blood films on slides. The Battlement method (C) was reported to produce the most accurate result.

Number of Cells to be Differentiated. In the differential leukocyte count, an attempt is made to determine the percentage distribution of the various leukocyte types in peripheral blood. The procedure is subject to considerable error since an extremely small portion of the total number of leukocytes is observed. The error can be decreased by counting a larger number of cells. Barnett¹⁶ in discussing the unavoidable error in the differential count, makes the following statement: "It is apparent that differential blood counts done on 100 cells, even with the most perfect technic and interpretation, are subject to such wide fluctuations as a result of chance variations in distribution that they are practically without significance. When to this unavoidable error there is added the mechanical error and that of interpretation, the result must necessarily be extremely unreliable. As has been repeatedly pointed out by others, at least 400 cells must be counted before the results of a differential count may be considered at all reliable and even here the maximum chance error may be as much as 7.5 per cent of the total count."

The differential count is time-consuming and, therefore, if a large total number of cells to be differentiated is rigorously insisted upon, this valuable procedure will be used to but a limited extent in veterinary

practice. On the other hand, to insure reasonable accuracy, the number of cells differentiated should be in proportion to the total leukocyte count. As a general rule, 100 cells should be differentiated for each 10,000 total leukocytes. Thus, for total counts of less than 10,000 leukocytes, 100 cells are differentiated; for total counts between 10,000 and 20,000, the total cells differentiated should be increased to 200; and, for counts above 20,000, the number of cells differentiated should be increased proportionately.

BLOOD EXAMINATION BY WET FILM METHOD

Considerable information about the formed elements of the blood can be obtained by examination of a wet film of blood under a coverslip. A stain such as that employed for the demonstration of reticulocytes may be added in equal volume with blood to make the leukocytes more distinct and reveal the presence of reticulocytes. The wet film method can be helpful in circumstances where more complete and critical blood studies cannot be made, or it may be employed as a rapid screening procedure on blood while the patient is on the examination table.

The procedure is useful for the detection of significant alterations in blood morphology but minor deviations from the normal are not readily demonstrable. After having acquired skill with the method, it is possible to ascertain the existence of anemia, leukopenia, leukocytosis, reticulocytosis, thrombocytopenia, lipemia, erythrocyte agglutination, abnormal morphology of erythrocytes, agglutination of leukocytes, abnormal blood debris, and certain types of blood parasites. The physiologic salt solution in which the stain is dissolved prevents to a considerable extent normal rouleau formation in canine blood but not in equine or feline bloods (Fig. 9-4). Immediate clumping of the erythrocytes in the wet film indicates that an elevated erythrocyte sedimentation rate may be anticipated.

Materials

1. Grease-free and lint-free microscope slides and 22 mm. square coverslips are required. Use new slides and coverslips; dip in alcohol and dry with lint-free cloth or facial tissue immediately before use.
2. Nichrome wire, 26 gauge, is formed into a loop having an outside diameter of 3 mm. or an inside diameter of just over 2 mm. The size of the loop must be such that it will hold the exact amount of blood or stain so that a mixture of one loopful of each will be adequate to spread evenly and completely under the cover-slip.
3. One per cent brilliant cresyl blue or 0.5 per cent new methylene blue dry stain* in 0.85 per cent saline is employed. Fungi may

*From Matheson Coleman and Bell Division, The Matheson Company, Inc., Norwood, Ohio or East Rutherford, New Jersey.

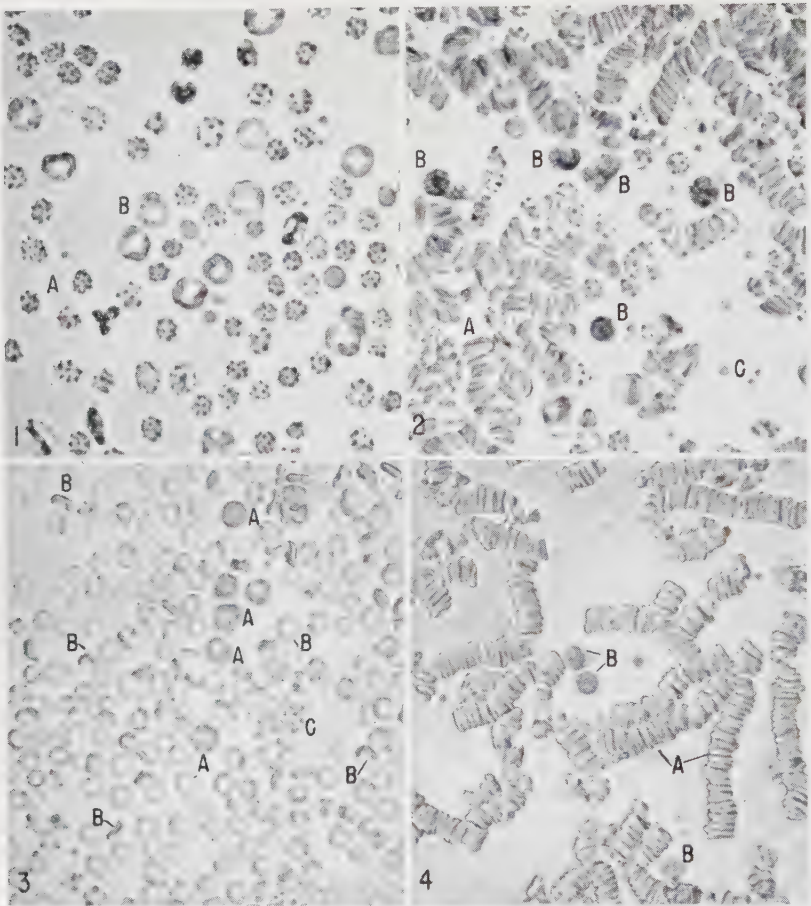


FIG. 9. Four examples of wet film preparations observed under high dry magnification. $\times 400$.

1, Canine blood in severe anemia with a marked reticulocyte response; A, crenated mature erythrocytes; B, all non-crenated, larger cells are reticulocytes.

2, Canine blood in a chronic infection; A, erythrocyte rouleaux with agglutination (anticipate increased erythrocyte sedimentation rate); B, leukocytes; C, thrombocytes.

3, Bovine blood in lymphocytic leukemic leukemia; A, lymphocytes; B, erythrocytes showing a tendency toward bowl-shape formation which leads to a "punched-out" center in the stained blood film; C, a cluster of thrombocytes.

4, Feline blood with a low normal volume per cent erythrocytes (PCV) which leads to extensive rouleau formation (erythrocytes stacked like coins). A, rouleaux; B, leukocytes.

grow in this stain and when this occurs the solution should be passed through filter paper. One ml. of formalin/100 ml. of stain may be added as a preservative.

4. Blood may be obtained directly from a free flowing drop from a punctured superficial vessel or collected via syringe and needle and transferred to a vial containing anticoagulant.

Preparation of the Film

1. The loop must be clean and dry. Clean the loop with facial tissue or cheesecloth immediately after the blood or the stain has been transferred to the cover-slip.
2. It is important to make certain that the loop is carrying the maximum quantity of blood or stain. With a properly filled loop, the fluid will appear biconvex or lens shaped.
3. The transfers are made to the cover-slip, depositing the stain first, followed by drying of the loop and then transferring the blood. The blood and stain are mixed by gentle circular movement of the loop but no attempt is made to spread the mixture over the cover-slip.
4. Blow on the slide to free the surface of dust or lint and then bring the cover-slip as close to the surface of the slide as possible and gently drop it onto the slide so that the film will spread between the glass surfaces. Occurrence of a large number of bubbles or failure of the film to spread under the entire area of the cover-slip will result in an unsatisfactory preparation.

Examination of the Wet Film

1. Place the film immediately under the high dry objective of the microscope and ascertain the degree of clumping of erythrocytes. Occurrence of irregularly shaped masses of tightly packed erythrocytes indicates that the erythrocyte sedimentation rate will be above normal. Clumping of the erythrocytes after five minutes is not as significant as its immediate occurrence (Fig. 9-2).
2. Count the leukocytes in ten high dry fields selecting areas not too close to the edges of the cover-slip. Record the result as an average number of leukocytes per field. The following statements are based on experience with 43 $\times$ objective and 10 $\times$ ocular. An average of 2 leukocytes or less per field would suggest leukopenia and an average of 13 or more per field would suggest total leukocyte counts of 20,000 or above. A differential count of leukocytes is not possible from the moist preparation. An alternate method for obtaining a stained blood film for purposes of differential leukocyte count consists of applying two loopsful of new methylene blue stain (0.5 per cent stain in 0.85 per cent saline solution) to an unfixed blood film on a cover-slip followed by dropping the cover-slip onto a slide, stain side down. The leukocytes stain immediately except that eosinophil-granules appear as unstained vacuoles. Erythrocytes do not stain. The mount is not permanent but is useful for several hours.
3. Occurrence of reticulocytes and the morphology of erythrocytes may be studied last; the reticulocytes stain more intensely and are more

easily seen as the moist preparation ages. Reticulocytes generally do not participate in formation of clumps of erythrocytes and thus they are usually observed to be distributed as isolated cells in the plasma. Erythrocytes of abnormal shape are readily detected in the wet film (Fig. 9-3).

4. The stained moist preparation presents an opportunity to make observations on the occurrence and relative number of thrombocytes (Fig. 9-2,3).
5. The moist preparation may reveal material in the plasma that is not seen in the stained blood film. This may be in the form of hyaline strands, crystals, amorphous material, or mirads of tiny vibrating specks (chylomicron, or emulsified fat).²⁵
6. Etiologic agents of disease may become apparent in the moist preparation. Bovine erythrocytes containing *Theileria mutans* have been seen and it may be that certain other types of erythrocytic parasites could be seen. *Haemobartonella felis* and *Anaplasma marginale* do not take the stain and cannot be seen in this type of preparation. When microfilaria are present in numbers of 1,000 or more per ml. of blood, these blood parasites may be found in the wet film preparation. The stain kills the microfilaria so that after a few minutes, movement of the parasite may stop.

THE WINTROBE HEMATOCRIT METHOD

The term "hematocrit" means to separate blood. By centrifugation, the blood is separated into three distinct compartments, namely: (a) the erythrocyte mass at the bottom, called the packed cell volume or PCV; (b) a white to gray layer of leukocytes and thrombocytes occurring immediately above the red cell mass and called the buffy coat; and (c) the blood plasma (Fig. 10).

The Wintrobe³¹ hematocrit tube has a uniform 3 mm. bore and is calibrated by a double 10 cm. scale with millimeter divisions. The scale on the left is read from top to bottom, while the scale on the right is in reverse. The volume per cent of each compartment of the blood, after adequate centrifugation, is determined directly by observing the height of each compartment in millimeters.

The anticoagulant must not distort the erythrocytes or an error will be introduced. For this reason, the balanced anticoagulant formula of Heller and Paul² is commonly used. However, the new anticoagulant EDTA is rapidly replacing the double oxalate formula for routine blood study (see anticoagulants). About 1 ml. of blood is required to fill the Wintrobe hematocrit tube and this is accomplished by means of a special pipette having a long, narrow, stainless steel delivery tip. The tip of the pipette is inserted to the bottom of the hematocrit tube and

as blood is forced out by pressure on the rubber bulb, the pipette is slowly withdrawn. When the column of blood reaches the level of the 0 mark on the left hand scale, the proper amount of blood has been deposited. While filling the tube, the tip of the pipette should remain

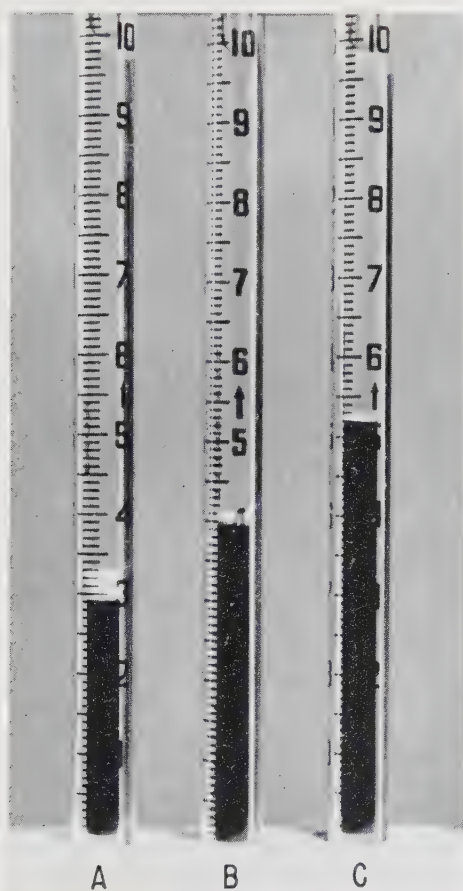


FIG. 10. Three different canine bloods in the Wintrobe hematocrit. A, the volume per cent erythrocytes or PCV is 29, which indicates *anemia*, and the white buffy coat is 4 mm., which indicates a marked *leukocytosis* (the actual total leukocyte count in this blood was 103,000 per cmm.). Tubes B and C are representative of canine bloods falling within the normal ranges for PCV and buffy coat.

just below the advancing level of blood in order to avoid the formation of bubbles. With a little practice, the hematocrit tube can be filled quickly and easily.

Sedimentation Rate of Erythrocytes. This test is especially applicable to canine blood and should be conducted routinely on all samples drawn

for purposes of the hematocrit test. The test should be started within the hour with oxalated blood, but may be delayed for as long as 6 hours when the anticoagulant is EDTA. The test is conducted by placing the hematocrit tube in a perfectly vertical position and observing the level to which the top of the column of erythrocytes has fallen in exactly 1 hour. Sedimentation is accelerated as the temperature rises and, therefore, in order to insure uniformity from day to day, the temperature of

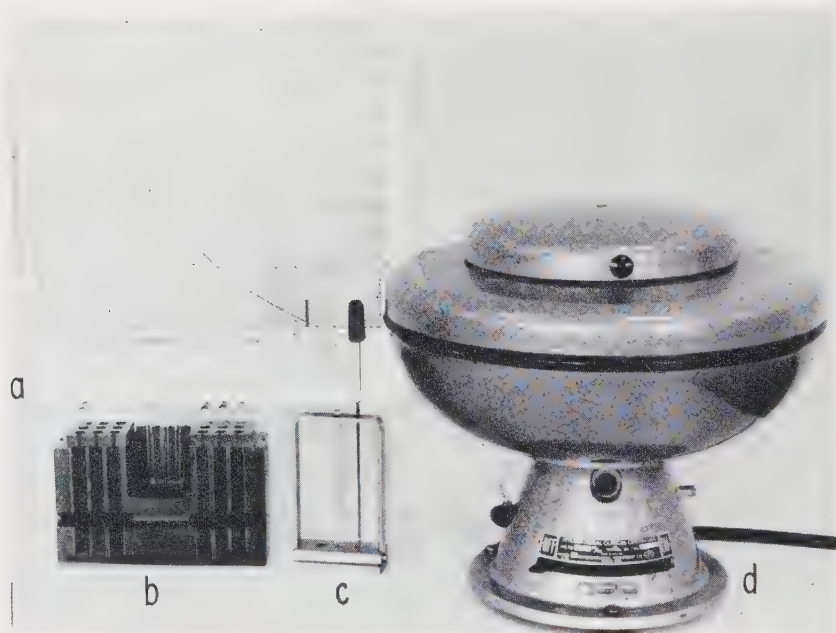


FIG. 11. The equipment for procedures with the Wintrobe hematocrit. A, erythrocyte sedimentation correction chart; B, icterus index standards and comparator block; C, metal rack for holding the hematocrit tube during the ESR determination and the capillary pipette for filling the hematocrit tube with blood; D, International Clinical Centrifuge which when operated at top speed is satisfactory for the hematocrit test.

the laboratory should not be permitted to be influenced too greatly by outside temperature fluctuations associated with season of the year.³⁰

The number of erythrocytes per unit volume of blood has a marked effect on sedimentation rate. The speed of settling is related inversely to the number of red cells. Thus, the smaller the number the greater the speed of settling. Since red cell number in health may vary over a wide range and in an individual animal the number of cells per unit volume of blood will vary from day to day with the state of water balance, it is important to compare the observed sedimentation rate with a standard or anticipated rate for the PCV in question. Wintrobe and

Landsberg³² have developed a correction chart for this purpose (Fig. 11). Data required are the observed sedimentation rate in mm., the PCV of the blood concerned, and a PCV representative of the mean for the species concerned. In the canine the correction is made to a PCV of 45, whereas in the cat a PCV of 35 is suggested. For correction of ESR in canine blood a table has been prepared of anticipated sedimentation in 1 hour for each PCV unit from 9 to 50 (Chapter 4, Table 11, p. 139). Since a difference of opinion exists as to whether or not correction is a valid procedure, both the observed rate and corrected rate should be recorded for each blood sample.

Detection of Reticulocytosis by Sedimentation Test. When many reticulocytes or young forms of erythrocytes are present, this becomes apparent from the sedimentation test. The reticulocytes do not enter into rouleau formation and, therefore, they trail-out in the plasma zone. This results in a hazy reddish plasma without a clear-cut line of separation between the settling red cell mass and the plasma.²⁹ Usually by the end of the hour the first mm. of plasma is clear, followed by the hazy reddish plasma which gradually merges with the main red cell mass (Fig. 12). This we have designated a diphasic sedimentation reaction. A diphasic ESR also may develop when the blood contains a large number of red cells of abnormal shape (Appendix, Case 12, p. 345).

The Hematocrit. The purpose of centrifugation is to obtain maximum packing of the erythrocytes so that little or no plasma remains trapped between the cells. A centrifuge is required that will produce sufficient relative centrifugal force (R.C.F.) to accomplish this purpose. Wintrobe⁷ states that maximum packing in human blood is obtained with a force of $2260 \times$ gravity for 30 minutes. To properly employ the Wintrobe hematocrit method, it is necessary to establish the conditions for obtaining $2260 \times G$ for a given centrifuge. The force obtained by centrifugation depends upon the radius at which a particle is distant from the center of revolution as well as the revolutions per minute. The radius is the distance in centimeters from the center of the shaft to the bottom of the hematocrit tube. The r.p.m. required of a given centrifuge can be determined according to Wintrobe by the following formula:

$$\text{r.p.m.} = \sqrt{\frac{202,146,700}{\text{radius in cm.}}}$$

The No. 2 International centrifuge with head having a 22.5 cm. radius will provide the necessary force at 3,000 r.p.m. The speed of the centrifuge is determined by means of a tachometer. The International Clinical Centrifuge, model CL, fitted with head number 213 and special metal shield number 301 produces a force of approximately $2260 \times G$ when operated at full speed or 3,800 r.p.m.

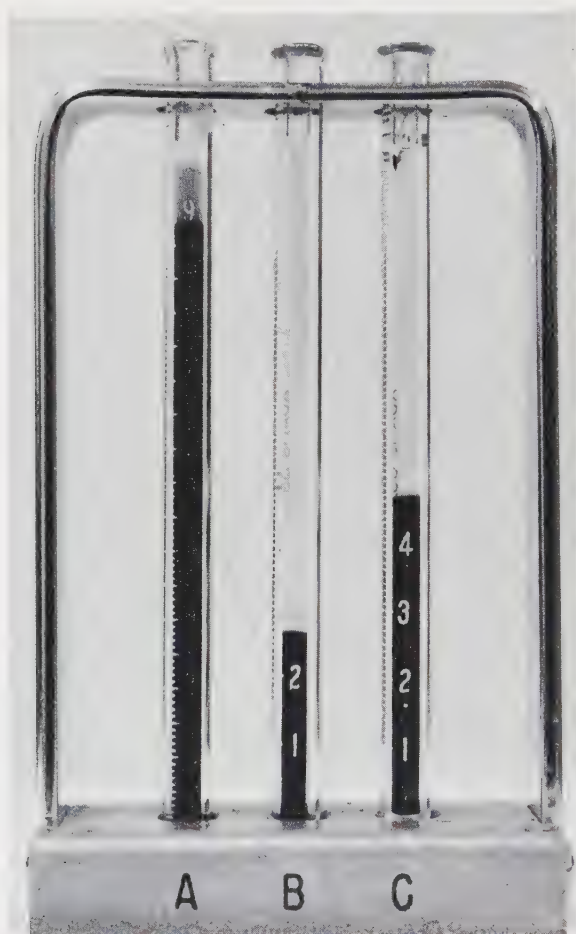


FIG. 12. A diphasic erythrocyte sedimentation rate in canine blood is shown in tube A. The same blood, after centrifugation for the hematocrit, is shown in B. Tube C contains a canine blood hematocrit test within the normal range for PCV for comparison with tube B. The hazy plasma zone of tube A reflects the trailing-out of reticulocytes during the 1 hour required for the erythrocyte sedimentation test. The PCV in tube B reveals an anemia to be present. Combining results of A and B it can be concluded that anemia exists and the bone marrow is responding with release of reticulocytes to peripheral blood. However, the latter assumption should be verified by wet film or stained blood film examination.

When the proper RCF is applied, satisfactory packing for routine study is accomplished in 30 minutes with canine and horse blood. Bunce²² states that with cattle and pig blood a centrifugation time of 60 minutes is required. A standard time of 45 minutes of centrifugation is satisfactory for routine hematology in the various animal species. Reference should be made to Chapter 1 for a discussion of the factors

to be considered in using the hematocrit in order to keep at a minimum the volume of plasma trapped among the red cells. When packing is complete, the PCV column has a translucent appearance. This phenomenon is called Koeppé's⁷ criterion for complete packing. It may be observed in anemia when the PCV is small and the erythrocytes are large. Koeppé's criterion is regularly seen in the PCV of the microhematocrit test (see page 61).

The Packed Cell Volume. The PCV is a function of erythrocyte size and number of cells per unit volume of blood. The hemoglobin content of the erythrocyte on a volume basis is between 30–35 per cent with a mean of 33 per cent, except in disease in which the utilization of iron is defective. For practical purposes of establishing a normal range for PCV in any species of domestic animal, the accepted normal range for hemoglobin may be multiplied by the number 3. For example, the normal hemoglobin range in the dog is generally accepted as 12–17 grams/100 ml. of blood; thus, the PCV range in mm. would be 36–51. However, since some plasma is trapped in the column of erythrocytes²⁴ to the extent of about 0.8 mm., between 30–40 mm. and 2 mm. at 50 mm. of PCV, the range for PCV in health in the canine should be stated as 37–53 mm. With this close relationship between hemoglobin content and PCV, it is possible to approximate the hemoglobin from the PCV by dividing the latter by 3. A factor of 6 when divided into the PCV gives a rough estimate of erythrocyte count in millions per cmm. of canine blood. These short cuts are intended only for approximation of hemoglobin and erythrocyte count in clinical medicine from the PCV where an exact figure is not always essential.

Anemia exists when the PCV falls below the minimum normal range for the species in question and hemoconcentration is present when the PCV exceeds the maximum of the normal range. However, it must always be kept in mind that an anemic animal in an extreme state of hemoconcentration may have a PCV in the normal range. Interpretation of the PCV must always be made in light of the water balance of the animal as determined by the physical examination (see Chapter 1).

The Buffy Coat. In blood from a normal animal, the buffy coat consists of a white to gray layer, 0.5 to 1.2 mm. in size, occurring immediately above the PCV. A delicate black line is often observed at the point of contact of the buffy coat and erythrocytes. This dark line is the result of action of the leukocytes upon the hemoglobin of the red cells whereby the oxygen is removed and reduced hemoglobin is produced. The thrombocytes are arranged at the very top of the buffy coat with the leukocytes below. According to Wintrobe each 0.1 mm. of the first 1.0 mm. is equal to 1,000 leukocytes, whereas each 0.1 mm. beyond the first 1.0 mm. is equal to about 2,000 leukocytes. Due to the fact that the number of thrombocytes is variable and the different leukocyte types

vary in size, it is not possible to predict accurately the total leukocyte count for any given sample of blood from the buffy coat. However, for routine clinical application, a buffy coat of less than 0.5 mm. would suggest leukopenia while above 1.5 mm. a leukocytosis is indicated.

In chronic diseases, erythrogenesis is often affected resulting in the production of erythrocytes of abnormal morphology. Such erythrocytes, as well as young forms of erythrocytes (reticulocytes), do not participate in the formation of rouleau and for this reason they tend to accumulate at the top of the erythrocyte column and may even be involved in the lower portions of the buffy coat where they impart a red tinge. Admixture of leukocytes and abnormal erythrocytes and/or reticulocytes in the buffy coat is enhanced when the sedimentation test for erythrocytes is conducted prior to centrifugation for the hematocrit test. Red-tinged buffy coats indicate that erythrogenesis has been affected by the disease process. When many reticulocytes are present, they not only produce a diphasic reaction in the sedimentation test but they may trap leukocytes during centrifugation so that the apparent buffy coat size is much less than would be anticipated by actual total leukocyte count on the same blood.

The Plasma Compartment. The volume of plasma normally exceeds 50 per cent. Bilirubin, a product of hemoglobin catabolism, imparts a yellow color to the plasma. The yellow color increases in hemolytic anemia, in certain types of liver pathology, and in bile duct obstruction. The degree of yellow pigmentation of the plasma is referred to as the icterus index. The icterus index is determined by matching the plasma color in the hematocrit with standards prepared from potassium dichromate and it is scored in units from 0 to 100.

In carnivorous animals such as the dog and cat, the yellow color is due entirely to bilirubin. In these animals, the icterus index is generally less than 5 units. A reading of 7.5 units in the dog or cat should be regarded with suspicion and over 7.5 units is definitely abnormal. In the cow and horse the yellow pigments carotene, carotenoid, and xanthophyll of plants and vegetables of the diet add additional color to the plasma and make the establishment of an upper limit for icterus index scores rather unsatisfactory. In addition, the horse has a higher bilirubin concentration than other domestic species^{23,28} and fasting for 48 hours causes a striking increase in plasma bilirubin. For these reasons, the icterus index score is not as useful in certain herbivores as in the carnivores. In general the normal icterus index score in the bovine is between 5 and 15 units and may go as high as 20 to 25 units when (*a*) the animal is on green feed or (*b*) in instances of acute disease with accompanying hemoconcentration. In the equine, icterus index scores of 7.5 to 20 units are common, but in one instance of what was considered to be a normal equine blood the score was recorded as 50. It is

important to take into consideration the type of feed and state of water balance of the animal when using the icterus index score in the cow and horse.

In a hemolytic disease in which the erythrocytes are being destroyed in the spleen by the reticuloendothelial system, bilirubin accumulates in the plasma and tissues due to inability of the liver to excrete the bilirubin as rapidly as produced. Under these conditions, the yellow color of the plasma increases. In certain diseases, intravascular hemolysis of erythrocytes may occur with development of hemoglobinemia. In such cases, the plasma is red due to the presence of free hemoglobin.

A turbid plasma indicates lipemia or the presence of a fine emulsion of fatty substance. In rare instances, a white opaque layer may appear at the very top of the hematocrit and this occurrence has been accompanied by hemoglobinemia. The hemolysis indicates the presence of a lytic agent in the plasma. The whitish opaque material may be soap produced from free fatty acids. Both soaps and fatty acids act as hemolytic agents.^{26,27}

THE MICROHEMATOCRIT

The advantages of the microhematocrit over the Wintrobe method are (1) more accurate and reproducible PCV values, (2) time required for the entire procedure is less than 5 minutes, and (3) amount of blood required is minimal making possible the use of the method on blood obtained by marginal ear vein puncture. The disadvantages are (1) a special reader is required to determine the hematocrit value and (2) due to the small size of the capillary tube, the erythrocyte sedimentation rate, the buffy coat value and the icterus index score are not as easily observed and determined as in the hematocrit method of Wintrobe. The microhematocrit is an accurate and practical procedure for measuring the venous or capillary hematocrit.³³

A comparison of the reproducibility of methods for measuring the erythrocytes revealed ± 0.5 per cent for the microhematocrit, ± 2.5 per cent for the Wintrobe hematocrit, ± 5.0 per cent for hemoglobin determination with the Klett photoelectric colorimeter, and ± 6.0 per cent for erythrocyte counts.³⁵ All tests were conducted under optimal conditions on human blood and the investigators concluded that under routine laboratory conditions the variations can be expected to be twice as great.

The microhematocrit method makes use of a high speed centrifuge (Fig. 13) which permits a corresponding reduction in the time required for complete packing of the erythrocytes. Time plus gravity required to produce complete packing with human blood was shown to be 1 minute at $28,000 \times G$; 2 minutes at $12,000 \times G$; 4 minutes at $8,000 \times G$; and 8 minutes at $4,000 \times G$.³⁶

Capillary tubes may be purchased with anticoagulant contained therein for filling directly from a punctured vein or plain capillary tubes are available for use on blood in a vial collected in the usual manner. The Drummond microhematocrit centrifuge employs capillary tubes measuring 32 mm. $\times$ 0.8 mm. After filling with blood between two-thirds to three-fourth capacity by capillary attraction, the outside is wiped off and the opposite end of the capillary tube is brought carefully to the edge of a blue gas flame near its base until it barely imparts a yellow or sodium color to the flame, and then the capillary tube is rotated be-

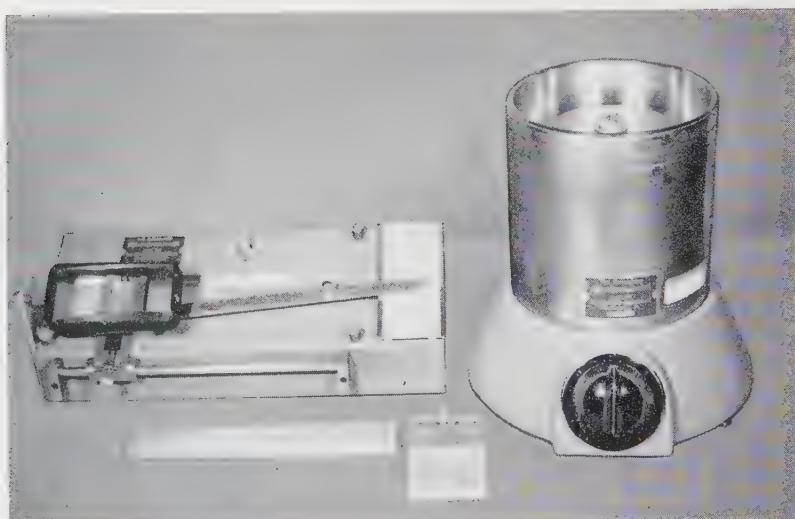


FIG. 13. Equipment for the microhematocrit. High speed centrifuge on the right and special reader on the left. In foreground there is a box of capillary tubes and the metal block for holding the tubes.

tween the thumb and index finger for 2-3 seconds. This seals the end of the tube and provides a flat inside closure with negligible distortion of the inside bore. The sealed capillary is centrifuged at 16,500 r.p.m. for 2 minutes. The International Hemacrit centrifuge employs somewhat larger capillary tubes, *i.e.*, 75 mm. $\times$ 1.0 mm. It has been stated³⁶ that the longer tubes require a longer period of centrifugation for complete packing of red cells and that the larger diameter may be associated with uneven bore. The blood is drawn into the tube by capillary attraction to a height of at least 2.5 cm. and the opposite end is sealed by heat as described above or by a plug of clay. Centrifugation is at 11,000 r.p.m. for 5 minutes. The capillary method using the 75 mm. $\times$ 1.0 mm. tubes has been adapted for use in the centrifuge employed for the Wintrobe hematocrit method, and centrifugation at $2260 \times G$ for 30

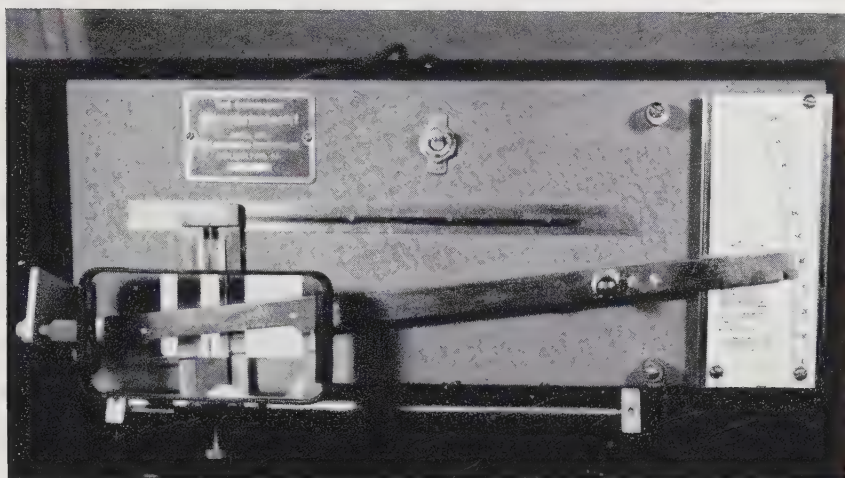


FIG. 14. The Drummond microhematocrit reader for use with capillary tubes measuring 32 mm. $\times$ 0.8 mm. The tiny capillary hematocrit can be observed as a narrow vertical black object in the illuminated rectangle of the lower left corner of the instrument. The PCV reading of 33 is noted on the scale at the right.

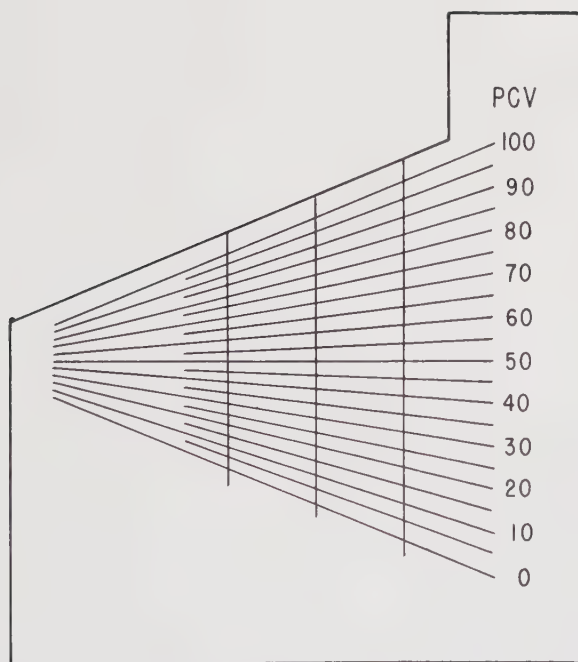


FIG. 15. Microhematocrit reader for use with capillary tubes 75 mm. $\times$ 1.0 mm. (from Kaneko³⁴).

minutes produced results with canine and feline blood having a correlation coefficient of 0.996 when compared with results obtained on identical blood samples in Wintrobe hematocrit tubes.³⁴ A short length of wooden doweling which fits snugly into 15 ml. centrifuge cups was prepared by making horizontal saw kerfs along the length of the dowel and deep enough to receive the 75 mm. $\times$ 1.0 mm. capillary tubes. Alternately metal carriers may be machined from aluminum rods.

The hematocrit value in the capillary tube is determined by use of a reader. The Drummond Microhematocrit reader (Fig. 14) is easy to use and a reader of this type is essential for the small 32 mm. capillary tube. For the 75 mm. tube a reader of the type shown in Figure 15 is used. The bottom of the red cell column is aligned with the bottom or 0 line and the tube is positioned so that the top of the plasma is aligned with the top or 100 per cent line. The hematocrit percentage is read from the right.

DETERMINATION OF HEMOGLOBIN

The hemoglobin molecule consists of protoporphyrin, native globin, and ferrous iron. It is a protein having a molecular weight of approximately 66,000. The iron content is 0.335 per cent or 3.35 mg./gram of hemoglobin. The oxygen capacity is 1.36 cc. per gram of hemoglobin. The most accurate methods for hemoglobin measurement are based on the chemical determination of iron content or oxygen capacity. These methods are too involved for practical clinical hemoglobinometry and, therefore, a number of rapid procedures have been developed based on (a) direct matching of the red color with artificial standards; (b) conversion of hemoglobin to acid hematin and matching the brown color with glass standards; (c) measurement of oxyhemoglobin by its light absorption in the green portion of the spectrum, using a suitable green filter and matching through an eyepiece with colored glass standards; or (d) measurement of light absorption of oxyhemoglobin, cyanmethemoglobin, or carboxyhemoglobin by means of the photoelectric colorimeter or spectrophotometer (Tables 3 and 4).

For a thorough review of the problems of measurement of hemoglobin, reference should be made to Symposium on Clinical Hemoglobinometry.³⁸ The following is quoted from this Symposium: "Despite the great numbers of hemoglobin measurements that are constantly being made and have been made for many years, *the analysis of hemoglobin remains one of the most unsatisfactory measurements in clinical use.* This, to a large extent, is due to the uncertainties encountered with the continuance of old colorimetric measurements that employ artificial standards for matching. Owing to the complexity of the absorption spectrums of hemoglobin and its derivatives, it is virtually impossible to devise artificial standards with the same colors as hemoglobin and its derivatives. . . . With

these methods, it may be said that the accuracy of the measurements is inversely proportional to their ease of manipulation."

Table 3. Methods Available for Clinical Hemoglobinometry³⁸

Type of Method	Remarks
Colorimetric	
1. Direct matching	1. Artificial standards not same color. Inaccurate.
2. Acid hematin	2. Erratic. Matching difficult with colored glass. Fading. Plasma constituents affect color.
3. Alkaline hematin	3. Less erratic than acid hematin but same difficulties.
4. Oxyhemoglobin	4. Simple, rapid. Fading. Failure to measure hemoglobin derivatives.
5. Pyridine hemochromogens	5. Precise, rapid. Fairly good color. Unpleasant odor.
6. Carboxyhemoglobin	6. Rapid, precise. Stable color. Requires source of carbon monoxide.
7. Cyanmethemoglobin	7. Rapid, precise. Measures hemoglobin derivatives. Employs cyanide reagents.
Physical measurements	
1. Specific gravity	(General statement for this group)
2. Hematocrit readings	Indirect. Not entirely specific for hemoglobin.
3. Refractive index	
Gasometric	
1. Oxygen capacity	Precise. Requires special equipment. Time-consuming. May not measure all hemoglobin derivatives present.
2. Carbon monoxide capacity	
Chemical	
1. Iron concentration	Precise. Time-consuming. Method of choice for checking and calibration purposes. Assumes non-hemoglobin iron to be negligible.

Table 4. Comparison of Results with Several Methods for Hemoglobin Determination on the Same Blood

Blood	Leitz ¹ Photo- meter	Coleman Jr. ¹ Spectro- photometer	Haden- Hausser (electric)	Haden- Hausser (daylite)	Spencer Hb-meter	Sahli- Adams #1 ²	Sahli- Adams #2 ²
Cow #1	14.3	14.0	11.8	12.5	14.0	12.0	13.0
Cow #2	14.3	13.8	12.5	13.0	14.4	12.8	13.0
Cow #3	8.6	8.9	8.5	9.0	8.6	7.0	7.5
Cow #4	9.0	9.0	8.5	8.0	8.8	7.0	8.0
Cow #5	7.4	6.8	7.8	7.5	6.4	5.5	6.0
Dog #1	8.0	8.4	8.0	8.5	8.0	6.5	7.0
Dog #2	13.0	13.25	11.5	12.0	12.6	11.0	11.5
Dog #3	15.0	14.4	13.0	14.0	14.5	13.0	13.0
Av. variation in gm./100 ml. from Coleman reading	+0.2	0	-0.8	-0.5	-0.1	-1.65	-1.2

¹ Oxyhemoglobin method.

² Two separate Sahli-Adams hemoglobinometers.

Direct Matching. Hemoglobin is red and the depth of color it imparts to the blood is directly proportional to the concentration of iron. As early as 1900, Tallqvist³⁹ proposed a method for evaluation of the quantity of hemoglobin in the blood by direct comparison of a drop of blood on blotting paper to lithographed color standards. In the same year, Dare³⁷ described a hemoglobinometer in which undiluted blood is placed in a capillary space between two pieces of glass and the color compared, through an eyepiece, with graded glass standards in a disk which can be rotated to obtain the best match. The errors with these methods are said to range from ± 10 to 40 per cent.

Hematin Methods. Sahli³⁸ introduced a method for hemoglobin determination which is used in various modifications such as the Sahli-Adams, Sahli-Haden, Haden-Hausser, and Newcomer hemoglobinometers.

A measured quantity of blood is treated with 1/10 normal HCl. The acid separates the globin from the hemoglobin molecule producing acid hematin having a brown color. The maximum color does not develop until 40 minutes have elapsed after which the color begins to fade. The various hemoglobinometers employing the acid hematin method use brown glass standards adjusted to color intensity to permit reading the result within a stated time period such as 1, 5, 10, or 30 minutes. It is important to make the final reading within the exact time period for which the instrument is adjusted to obtain greatest accuracy. By careful technic, the error in acid hematin method may be held to ± 5 to 10 per cent.

Wu⁴¹ found that the errors of the acid hematin method resulting from variation in content of proteins and lipoids in the plasma in disease may be overcome by converting the acid hematin to alkaline hematin by the addition of sodium hydroxide. This suggestion has not been followed in the acid hematin methods available to the veterinary practitioner.

Oxyhemoglobin, Direct Method. The Spencer Hb-meter measures oxyhemoglobin by light absorption using a green filter. A drop of blood is placed in a shallow space of a glass plate and laked by a hemolytic agent present on the tip of an applicator stick. The proper thickness of laked blood is obtained by placing a second piece of glass on top of the chamber and pressing the two glasses together. The glass chamber is inserted into the hemoglobinometer and the green color matched with the standard. The Spencer Hb-meter has the slight advantage of comparing green colors rather than brown. Maximum visual sensitivity to light occurs in the green range of the spectrum; also, the maximum absorption of hemoglobin occurs for visual light in the green band of the spectrum.

Photoelectric Colorimeter and Spectrophotometer Methods. These instruments are expensive but they have the distinct advantage of speed and accuracy and may be used for a number of other chemical determina-

tions. However, each instrument in use should be calibrated from time to time for hemoglobin determination against the iron measurement method of Wong.⁴⁰

The measurement of hemoglobin in the form of oxyhemoglobin is widely used. A 1–200 dilution of blood is prepared in either a 0.1 per



FIG. 16. Automatic pipette which is self-filling and self-setting at the zero point for accurately dispensing 5 ml. aliquots of cyanide reagent for hemoglobin determination.

cent solution of sodium carbonate or a 0.04 per cent solution of ammonium hydroxide. The latter is preferred since less fading of color takes place if the reading is not made immediately. The oxyhemoglobin solution is placed in the instrument and light absorption determined at a wavelength of 545 $m\mu$. The hemoglobin value is determined from a calibration curve that has been prepared from the hemoglobin measurements by iron analysis.

Carboxyhemoglobin and cyanmethemoglobin are derivatives that may be used for even more accurate hemoglobin measurement than can be obtained with oxyhemoglobin. A disadvantage of the carboxyhemoglobin method is that a source of carbon monoxide is required and with the cyanmethemoglobin a poisonous cyanide reagent is used. For details of a convenient method for cyanmethemoglobin determination, the reader is referred to Ortho Pharmaceutical Corporation, Raritan, New Jersey. For safety and accuracy with the cyanide reagent an automatic pipette which is self-filling and self-setting (Fig. 16) at the zero point is recommended. The pipette is calibrated to deliver accurate 5 ml. aliquots. In addition to the safety feature, the apparatus permits rapid and accurate dilution of blood for hemoglobin determination.

ENUMERATION OF ERYTHROCYTES AND LEUKOCYTES IN THE HEMOCYTOMETER

The hemocytometer consists of a red cell diluting pipette, a white cell diluting pipette, and a counting chamber. When purchasing the hemocytometer or in replacement of parts, it is advisable to select equipment claimed by the manufacturer to meet the specifications for accuracy of the National Bureau of Standards. Pipettes and counting chambers certified by the National Bureau of Standards may be obtained by paying an additional sum to cover the cost of testing and certification.

Filling the Pipette. The diluting pipette consists of a calibrated stem portion and a bulb or mixing chamber containing a glass bead to facilitate uniform dispersion of the cells in the diluting fluid. The stem portion is divided by marks into 10 equal parts, the total of which is one unit. On the opposite side of the bulb, the number 11 appears on the white cell pipette and the number 101 on the red cell pipette. The volume of fluid required to fill a pipette to the upper mark does not represent a specific amount which is the same for all pipettes. The marks on each individual pipette, however, indicate comparable volumes for that pipette. Therefore, it may be said that the calibrations represent comparable volumes of no specific amount.

A rubber tube fitted with a glass or plastic mouthpiece is attached to the upper end of the pipette. By gentle suction, blood is drawn into the stem to the mark desired. In routine blood work, this is to the 0.5 mark for both leukocyte or erythrocyte counts. When the column of blood reaches the 0.5 mark, the lips are parted slightly to permit atmospheric pressure to reduce the vacuum in the oral cavity and this stops the flow of blood into the stem of the pipette. If blood is drawn beyond the 0.5 mark, the excess may be removed by touching the tip of the pipette with the finger and the desired amount of blood can be withdrawn by capillary action. The tip is wiped clean and then the proper diluting fluid is

drawn into the pipette to exactly the 11 mark or 101 mark of the respective instrument. As the diluting fluid enters the pipette, the blood moves into the mixing chamber. During this process, the pipette stem should be rolled back and forth between the thumb and index finger to facilitate mixing of the blood with the diluting fluid. To avoid an error in dilution, the fluid should be stopped exactly at the upper mark.

The white cell pipette gives a dilution of 1-20 and the red cell pipette a dilution of 1-200 when blood is drawn to the 0.5 mark and diluted; 0.5 volume of blood is diluted with 9.5 or 99.5 volumes of fluid in the white and red cell pipette, respectively (Fig. 17). Greater or lesser

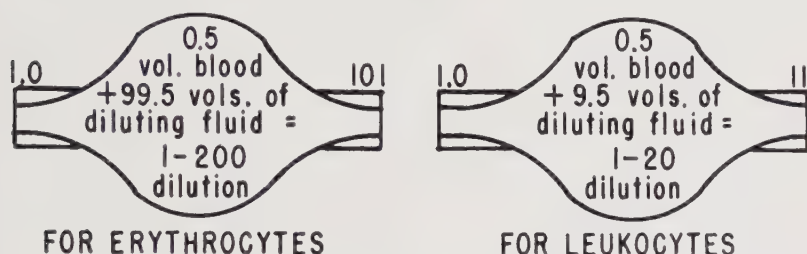


FIG. 17. In the counting of the red and white blood cells, the appropriate blood diluting pipette is employed and blood is drawn into the capillary portion to the 0.5 mark. The proper diluent is then drawn into the pipette and this moves the blood from the stem into the bulb of the pipette where it becomes diluted as shown above.

dilutions may be accomplished by drawing blood to levels other than the 0.5 mark. In extreme anemias, the white cell pipette may be employed for erythrocyte count; and, in blood having exceedingly high leukocyte numbers, the red cell diluting pipette may be used.

The diluting fluid for the white cell count consists of an acid to lyse the erythrocytes. Either 1 per cent HCl or 2 per cent acetic acid may be used. One ml. of a 1 per cent aqueous solution of gentian violet may be added to each 100 ml. of leukocyte diluting fluid to distinguish it from other solutions employed in the laboratory.

The diluting fluid for the red cell count should be isotonic for erythrocytes. Hayem's solution, Gower's solution, or 0.85 per cent saline is commonly used.

Hayem's solution

Mercuric chloride	0.5 Gm.
Sodium sulphate, crystals (or anhydrous, 2.2 Gm.)	5.0 Gm.
Sodium chloride	1.0 Gm.
Distilled water	200.0 ml.

Gower's solution

Sodium sulfate	12.5 Gm.
Glacial acetic acid	33.3 ml.
Distilled water	200.0 ml.

Physiologic saline

Sodium chloride	0.85 Gm.
Distilled water	100.0 ml.

Transfer a small quantity of the stock red and white cell diluting fluids to shell vials. Fill pipettes from the shell vials and replace the solution in the vial as soon as the slightest sediment or cloudiness appears.

Hayem's solution may cause agglutination of erythrocytes in some bloods. This is due to the action of the mercury salt on the protein of the plasma. Agglutination is especially prone to occur with bovine, sheep and goat blood. Gower's solution has the advantage of preventing agglutination in most bloods, but in instances where agglutination does occur with either Hayem's or Gower's, it can usually be prevented by use of physiologic saline as the diluent. A disadvantage of Gower's is that diluting pipettes must be cleaned often, preferably after each use, because of a brownish deposit left in the pipette with continued use of the solution. Hypochlorite cleaning solution (chlorox) removes this simply by rinsing.

The Counting Chamber. The counting chamber consists of a rectangular piece of heavy glass presenting two raised cross bars on which the cover-slip rests. In the central area between the cross bars are two platforms, each of which is completely surrounded by a moat. The polished surface of each platform is 0.1 mm. below the cover-slip so that when the chamber is filled the depth of fluid is 0.1 mm. Each platform presents a ruled area consisting of 9 primary squares, each of which encompasses 1 square mm. Each of the 4 corner primary squares is subdivided into 16 secondary squares for purposes of facilitating the counting of the leukocytes. The center primary square is used for the erythrocyte count and it presents 25 secondary squares, each of which is further subdivided into 16 tertiary squares. The total number of tertiary squares in this central area is 400. The borders of the secondary squares of the red cell counting area are made up of two or three parallel lines to facilitate selection of erythrocytes to be included in the count. It is customary to enumerate all erythrocytes in five of the secondary squares, using the 4 corners and the center square.

Before filling the chamber, the pipette should be shaken for 2 minutes to bring about a uniform dispersion of the blood cells in the diluting fluid. The pipette is held by placing the thumb over the tip and the index or middle finger over the opposite end. Shaking is accomplished by a back and forth movement of the pipette at right angle to its long axis. If shaken in the same direction as the long axis, cells may be forced from the mixing chamber into the stem and thereby an error could be introduced. Blow out and discard up to one-half the contents of the pipette to remove the cell free fluid from the stem; touch the tip of the pipette

to the edge of the platform of the counting chamber and allow the fluid to flow under the cover glass by capillary action. The filling of the chamber should be accomplished by uniform movement of the fluid across the surface of the platform. The amount of fluid should be controlled so as to just fill the chamber without spilling into the moat. Fill one side of the chamber from the white cell pipette and the other side from the red cell pipette. Count the leukocytes first, thus permitting sufficient time for the erythrocytes to settle to the surface of the platform.

Counting the Cells and Calculations. The low power objective is used for the white cell count. With a 16 mm. lens and 10× eyepiece, the field is filled by the area of one primary square. It is customary to count the leukocytes in the four corner primary squares and multiply by a factor of 50 to obtain the number of cells in 1 cmm. of blood.

Fluid in each primary square of the chamber	= 1/10 cmm.
Four primary squares	= 4/10 cmm.
4/10 × 1/20 (dilution factor)	= 1/50 of cmm. of undiluted blood

When nucleated erythrocytes are present, they appear in the total leukocyte count. The number of nucleated cells encountered while making a differential count of 100 leukocytes in the stained blood film is noted and employed to correct the leukocyte count as follows:

$$\frac{100 \text{ leukocytes}}{100 \text{ leukocytes} + \text{number nucleated erythrocytes}} \times \frac{\text{uncorrected leukocyte count}}{\text{count}} = \text{corrected leukocyte count}$$

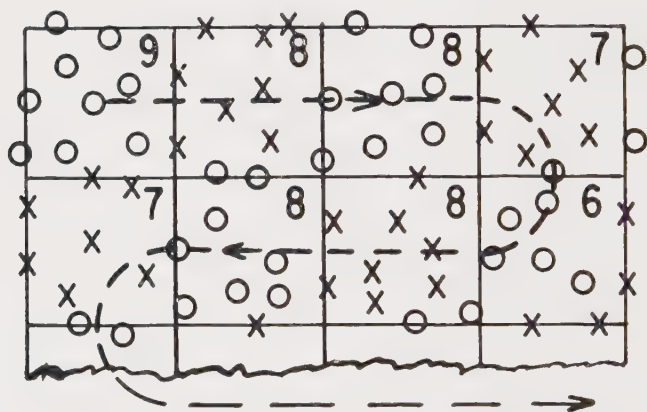
The high dry objective is employed in making the erythrocyte count. The number of cells in 5 secondary squares (80 tertiary squares) is determined and multiplied by 10,000. This is accomplished by merely adding four zeros to the total number of cells, and this represents the erythrocyte number/cmm. of blood.

$$1/5 \text{ sq. mm. area counted} \times 1/10 \text{ mm. in depth} \times 1/200 \text{ dilution} = 1/10,000 \text{ of cmm. of undiluted blood.}$$

A definite system for counting must be followed to avoid missing cells or counting cells more than once (Fig. 18). The usual practice is to start at the upper left square and include in the count for each respective square all cells touching the lines forming the upper and left borders and ignoring cells touching the bottom and right border lines. Move across the top row of squares from left to right and then drop down to the second row and proceed from right to left. Repeat this meander pattern until all squares have been covered as required for either the white cell or red cell count.

Inherent Errors in the Blood Cell Count as made with the Hemocytometer. Berkson, Magath, and Hurn⁴² have investigated the normal error of the count or inherent error in the procedure. Errors arise for the following reasons:

1. The cell count is estimated on a very large volume with a very small sample.
2. The random settling of cells in the counting area of the chamber, even in properly mixed samples, is subject to chance. This is referred to as the "error of the field."
3. Filling separate counting chambers leads to variations in count. This is called the "error of the chamber."
4. The filling of different pipettes leads to variations in count; called the "error of the pipette."



Cells on lines forming left and upper borders are to be included in the count for that square; cells on lower and right borders are ignored until contiguous squares are counted.

FIG. 18. The recommended pattern for counting blood cells in the hemocytometer chamber.

The total error inherent in the procedure for a mean count of 5,000,000 erythrocytes/cmm. would be 7.8 per cent at the level of one standard deviation. However, in routine statistical practice, it is common to regard an estimate as significantly determined within ± 2 standard deviations, for 95 per cent of random counts will fall within these limits. Thus, the estimated count should actually be stated as 5,000,000 ± 16 per cent. The same authors state that for a leukocyte count of 7,000 obtained by counting 4 sq. mm., the count is determined as significant within ± 21 per cent.

An experienced technician can obtain erythrocyte counts on the same blood sample showing surprisingly little variation. However, when the

cell counts of two trained persons are compared on the same blood sample, a significant difference in results may be anticipated.³⁵

When conducting critical studies, as in research, where erythrocyte enumeration is involved, it is important to have the same technician make all counts throughout the study in order to limit variation as much as possible.

ENUMERATION OF EOSINOPHIL LEUKOCYTES

The direct counting of eosinophils is of importance in evaluating adrenocortical activity. In the presence of a normal adrenal gland, the eosinophil count in peripheral blood decreases upon administration of adrenocorticotrophic hormone (ACTH) or cortisone.^{43,45} Thus, the direct counting of eosinophils following injection of ACTH has been suggested as a screening test for adrenocortical insufficiency.^{46,48} A case of primary adrenocortical insufficiency in a 4-year-old dog has been reported in which administration of ACTH failed to elicit an eosinopenic response.⁴⁷

A diluting fluid for direct eosinophil counts employs propylene glycol to lyse erythrocytes and sodium carbonate to lyse all leukocytes except the eosinophils.^{48,49} It is prepared as follows:

Propylene glycol	50 ml.
Distilled water	40 ml.
Stock solution consisting of 1% phloxine in distilled water	10 ml.
Stock solution of 10% sodium carbonate	1 ml.

Careful measurement of the sodium carbonate is necessary. Mix and filter after which the solution is stable at room temperature for at least one month. The dilution fluid stains the eosinophils red. (Farrington and Jeffer⁴⁴ have described what they call an improved staining solution for counting eosinophils in dog blood.)

The direct eosinophil count is made by drawing blood to the 1.0 mark into the white cell pipette followed by the special diluting fluid being drawn to the 11 mark. The pipette is shaken for 2 minutes, the diluting fluid in the capillary portion is discarded and both chambers of two hemocytometers are filled for counting. Wait 15 minutes for complete lysing of all cells before making the eosinophil count. To increase accuracy it is recommended to fill two diluting pipettes and a total of 8 chambers on 4 hemocytometers and the eosinophils in all 9 squares of each chamber are recorded. The 9 primary squares of a single counting chamber total 0.9 mm.³ The dilution of blood in the pipette was 10 $\times$, therefore $1.0 \div 0.9 \times 10 = 11.1$. The average number of eosinophils per hemocytometer chamber $\times 11.1 =$ the number per cubic millimeter of blood.

MEASUREMENT OF MEAN ERYTHROCYTE SIZE

The size and hemoglobin content of the erythrocyte are useful in the morphologic classification of the anemias. The size of the red cell and volume of hemoglobin contained therein may provide clues to the probable cause of an anemia. Thus, a microcytic hypochromic red cell is characteristic of anemia due to iron deficiency or faulty utilization of iron; normocytic normochromic red cells are characteristic of health but also are found in anemia resulting from depression of erythropoiesis; and, macrocytic erythrocytes are characteristic of the response to replace red cells after blood loss or anemia associated with maturation arrest in the early stages of erythropoiesis.

Direct Measurement of Erythrocyte Diameter. Price-Jones^{52,53} developed an accurate but time-consuming method for obtaining the mean diameter of the erythrocyte and for demonstration of the scattering of red cells by size within a given blood sample. The images of the erythrocytes in a stained film are projected on paper at a magnification of exactly 1,000 times. The erythrocytes are outlined in pencil on the paper and the diameters of at least 500 cells determined with a millimeter rule. Since each mm. contains 1,000 microns and the red cells are magnified 1,000 times, the results are recorded directly in microns; the distribution is designated the Price-Jones curve. A modification of the original method is the direct measurement of erythrocytes with an eyepiece micrometer which has been calibrated by means of a slide micrometer scale.

Diffraction Methods for Obtaining Mean Diameter of the Erythrocyte. Thomas Young,⁵⁰ in 1813, was the first to apply the principle of diffraction to the measurement of spherical objects including the human erythrocyte. The observations by Young were forgotten; and, in 1918, Pijper⁵¹ independently rediscovered the method. He found that a beam of white light undergoes diffraction at the edges of spherical objects, producing colored rings arranged in spectrum fashion. A formula was developed to permit calculating the diameter of the spherical objects from the diameter of the diffraction spectra. The principle of the diffractometer depends on the fact that the size of the spectrum varies with the size of the red cell and its distance from the light source.

The Haden-Hausser erythrocytometer⁵⁰ (Fig. 19) consists of two cylinders, one of which telescopes into the other. By means of a rack and pinion, the outer cylinder is caused to move up or down thus varying the distance between the top of the outer cylinder and the bottom of the inner cylinder. A 50 watt electric bulb is placed in the base and a metal disk is inserted across the bore of the inner cylinder. This disk has a small central aperture to emit a beam of light for use in producing the spectrum and in addition the disk contains three sets of smaller holes,

not large enough to create separate spectra. At the top of the outer cylinder is a slot for holding the slide containing the blood film. As the film is caused to move away from the light source, the colored rings of the spectrum become larger. The distance of the blood film from the light source is adjusted until the inner red ring of the spectrum directly



FIG. 19. The Haden-Hausser erythrocytometer for measuring mean red cell diameter in an unstained, dry blood film by the principle of light diffraction.

overlies the inner circle of small holes. The mean size of the erythrocytes in the film in microns is read directly from the calibration on the outer rim of the wheel used to move the outer cylinder up and down.

A dry unstained blood film is used. The film must be thin enough so that the erythrocytes do not overlap and yet not so thin that the cells have spread-out increasing their diameter. The slide should be placed in the slot and moved across the beam of light until the area where the

spectrum is sharpest is found. Then match the inner red ring with the inner set of apertures. In a blood sample consisting of erythrocytes of essentially the same size, the second set of small holes will fall in the yellow zone and the outer set will match the outer red zone. Where considerable variation in red cell size occurs, the middle and outer sets of holes will not fit perfectly over the yellow and outer red, respectively. Each cell throws its own corona, so the point of maximum intensity corresponds to the figure representing the diameter of the greatest number of cells.

THE WINTROBE ERYTHROCYTIC INDEXES

The PCV or packed cell volume of the hematocrit, the number of erythrocytes per cmm. of blood, and the hemoglobin in grams per cent are the basic data required for calculation of these indexes.^{54,55} The validity of the indexes is influenced by the accuracy of the red cell count, the hemoglobin determination and the hematocrit value. In obtaining the hematocrit value, it is important to employ a technic that reduces to a minimum the volume of plasma trapped in the red cell column. Significant trapping of plasma is especially prone to occur with cow, sheep, and goat bloods because of small red cell size and absence of rouleaux. The microhematocrit method provides a more accurate PCV with such bloods than the Wintrobe hematocrit.

Mean Corpuscular Volume. Given a PCV of 42 per cent and an erythrocyte count of 6 million, the MCV would equal 70 cubic microns.

$$1 \text{ mm.} = 1000 \text{ microns}$$

$$1 \text{ mm.}^3 = 10^9 \text{ cubic microns}$$

$$42 \text{ per cent of } 10^9 \text{ cubic microns} = 420,000,000 \text{ cubic microns}$$

$$\frac{420,000,000}{6,000,000} = 70 \text{ cubic microns}$$

In the interpretation of the MCV, it must be kept in mind that it is based on the erythrocyte count which in itself is subject to considerable error. However, as pointed out in the discussion of the error in the erythrocyte count, a well-trained technician is able to reproduce counts on the same blood sample with little variation; but significantly different results may be anticipated when two or more technicians make erythrocyte counts on the same blood. Thus, as with erythrocyte counting, mean normal MCV for the various animals will be influenced by the personal equation of the technician doing the work. For example, if a 10 per cent $\pm$ error existed between the erythrocyte counts obtained by two or more technicians on a canine blood sample having a PCV of 42 per cent and a mean RBC of 6.0 millions, the MCV would fall any place between 63 and 77 cu. In use of the MCV in clinical medicine, it is

necessary to establish the normal mean for the species as determined by the technician doing the work.

Mean Corpuscular Hemoglobin. This index expresses in micromicrograms the weight of the hemoglobin in the average erythrocyte of a population of cells.

One gram hemoglobin = 10^{12} micromicrograms ($\mu\mu\text{g.}$).

Hemoglobin is reported in grams/100 ml. of blood.

RBC in millions/cmm. of blood must be converted to the number/100 ml. of blood.

Given 14 grams hemoglobin and 6.0 million RBC, the MCH = $23.3 \mu\mu\text{g.}$

$$\frac{14 \times 10^{12}}{100 \text{ ml.} \times 1,000 \text{ cmm.} \times 6,000,000} = \frac{14,000,000,000,000}{600,000,000,000} = \frac{140}{6} = 23.3 \mu\mu\text{g.}$$

Mean Corpuscular Hemoglobin Concentration. This index expresses the volume per cent of the erythrocyte occupied by the hemoglobin. It measures the ratio of weight of the hemoglobin to the volume of the erythrocyte and the expression is in per cent. In health, the MCHC ranges between 30 and 35 per cent for all species of domestic animals. The MCHC result is seldom valid when it is above 35 per cent, for the maximum amount of hemoglobin that can be held by a red cell perhaps is not much greater than 35 per cent.* On the other hand, the hypochromic anemias are characterized by the MCHC falling below 30 per cent. When the method for obtaining the hematocrit value fails to minimize the trapped plasma, a lower MCHC index is obtained in the calculation. This is apt to occur with cow, sheep and goat blood when PCV is based on the Wintrobe hematocrit and especially when the red cell volume is large.

$$\frac{\text{Hemoglobin gm./100 ml.}}{\text{PCV in ml./100 ml.}} \times 100 = \% \frac{14}{42} \times 100 = 33.3\%$$

ASPIRATION OF BONE MARROW FROM THE ILIAC CREST

The primary function of bone marrow is the production of erythrocytes, granulocytes, and thrombocytes. Bone marrow which is active in this respect is red in color and is called red marrow whereas non-productive resting marrow is called yellow marrow. The latter consists of three types of cells; namely, endothelial, reticular, and fat cells. In the adult, the yellow marrow fills most of the shaft of the long bones. Demands for increased production of blood cells are met by conversion of yellow marrow into red marrow. Red marrow expansion or regression is made possible through interchangeability with fat cells. The fat cells serve as a space-holding mechanism and they readily give up the space to red marrow or occupy it again on red marrow shrinkage.

*Since the introduction of the microhematocrit method, which produces a PCV with less trapped plasma, the MCHC may exceed 35 per cent at times when the determination is based on PCV from the microhematocrit.

The flat bones, such as the ribs, pelvis and bones of the head, the short bones, such as the vertebrae, and the ends of the long bones normally contain red marrow throughout life. To obtain bone marrow fluid for study from the living animal it is necessary to select a site for puncture of the marrow cavity that is readily accessible. In man, the sternum is most frequently used and may be used in animals. However, in most animals, the iliac crest has been found to be a more convenient site. This is an especially convenient site for marrow aspiration in the dog and cat and may be employed in the large domestic animals. However, with aged cows and horses, the red marrow may recede beyond the point of accessibility in the ilium; in that event, penetration into the marrow cavity of a rib may become necessary.

Meyer and Bloom⁵⁷ have described the technic for obtaining bone marrow from the iliac crest of the dog, Sawitsky and Meyer⁵⁸ described the procedure for the cat, and Archer⁵⁶ for the horse. For the canine, Meyer and Bloom recommend a 1¼ inch, 15 to 18 gauge Kaliski needle with attached stylet; Sawitsky and Meyer suggest, for use on cats, a 1½ inch, 18 to 19 gauge spinal tap needle with straight stylet. Ordinary bleeding needles may be employed but the sharp point should be ground down to give a more blunt and stronger end for penetration of the bone.

Technic. The iliac crest is outlined with the finger, the hair is clipped in the immediate site of the puncture, usually the anterior-dorsal angle, and a suitable antiseptic is applied. Although Meyer and associates state that local anesthesia is not required, it is best to infiltrate the area with 2.0 ml. of 2 per cent procaine. Make a short slit in the skin with a sharp scalpel to facilitate penetration of the skin by the needle. An assistant may hold the dog in a standing position but, if the animal tends to struggle, it should be placed on its side with the hind legs extended or in a dorsum-up position with the hind legs held tightly tucked under the abdomen.

The sterile aspiration needle is passed through the skin and muscle over the anterior-dorsal angle of the crest of the ilium. Upon reaching the periosteum, the needle is forced into the bone by steady pressure and back and forth rotation. When the needle is firmly embedded, it has usually penetrated the medullary cavity. The stylet is used to free the needle of tissue or bone particles. A dry 20 ml. glass syringe is fitted to the needle and the plunger pulled out a considerable distance to establish sufficient vacuum to withdraw marrow fluid. As soon as fluid appears, the suction process should be stopped or otherwise dilution of the marrow fluid with blood may occur. Remove the syringe and needle and deposit the marrow fluid on a clean slide. If a large drop has been obtained, a number of smears may be made by touching the edge of a clean slide to the drop and quickly spreading the marrow fluid on the surface of other slides. The films are dried quickly by waving

in the air and staining is accomplished by the usual method for staining blood films.

A differential count is made of 1,000 nucleated cells. A bone marrow evaluation form carrying the names of all cell types to be differentiated is convenient for scoring the cells as observed and for recording total cells of each type. The M:E ratio is determined by dividing the number of nucleated red cells into the sum of all cells belonging to the granulocytic series. A myeloid-erythroid ratio of 1.0 would mean that nucleated cells of both series are present in equal numbers and, a M:E greater than 1.0 means that the granulocytic series exceeds the erythrocytic. Interpretation of the M:E ratio as applied to the anemias must be made in light of the total leukocyte count. When the total leukocyte count is within normal limits, a M:E less than 1.0 means intensification of erythropoiesis while a M:E greater than 2.5 reflects depression of erythropoiesis.

RAPID TESTS FOR HEMOSTATIC DEFECTS

Coagulation defects in animals are not of common occurrence and in general may be said to be limited to a few special instances, *e.g.*, sweet clover poisoning in cattle (Dicoumarol), bracken fern poisoning in cattle, trichloroethylene-extracted soy bean oil meal poisoning in cattle, poisoning from Warfarin (rat poison) in dogs, prolonged bleeding time in dogs associated with infectious hepatitis, rare cases in the canine of intestinal bleeding associated with liver cirrhosis (lack of prothrombin due to failure of absorption of Vitamin K) and the remote possibility of bleeding in the canine associated with a defect in vitamin C production (Appendix, Case 24).

Roswell *et al.*^{63a} have described a sex-linked recessive character leading to a bleeding disorder in male dogs of the Cairn Terrier breed. The disease is similar genetically and pathologically to hemophilia B or Christmas disease in man. It is characterized by a defective blood coagulation mechanism, due to a serum factor that affects thromboplastin formation. Several references are cited relative to the problem of hemophilia in the canine.

The mechanism of blood coagulation is a complex series of reactions which has as its essentials: (1) formation of thromboplastin from precursors in the blood platelets, (2) the conversion of prothrombin to thrombin by this active thromboplastin in the presence of calcium ion and, (3) the conversion of fibrinogen to fibrin by thrombin. In the diagnosis, treatment and study of hemorrhagic diseases, a number of tests designed to estimate the speed of coagulation and the presence or absence of the various factors involved in coagulation are available. A selected number of these tests may be employed as screening tests which may guide the clinician in further study, diagnosis or treatment. Pro-

cedures dealing with estimation of prothrombin activity are beyond the scope and purpose of this volume.^{62,63}

Bleeding Time. A moderately deep skin puncture (or mucous membrane) is made with a No. 11 Bard-Parker blade so that a free flow of blood is obtained. Pressure should not be used to obtain the free flow. At 30-second intervals, the drops of blood are removed by absorbing with a piece of filter paper and the time at which bleeding ceases is noted. In the domestic animals, the bleeding time varies between 1-5 minutes by this method.

Coagulation Time. Variation in bleeding times and coagulation times in normal animals are usually the result of variations in technique and temperature. Of these, temperature is of the utmost importance, particularly in coagulation time determinations, and it cannot be stressed too strongly that normal controls should be run together with the blood of the patient.

The coagulation time determination may be performed easily and quickly by either of two procedures. In the capillary method, a skin puncture is made similar to the bleeding time method. After wiping off the first drop of blood, blood is taken up into a capillary tube about 15 cm. long by 1-1.5 mm. in diameter. At intervals of 1 minute, the tube is broken off at 1-2 cm. pieces. When coagulation occurs, fibrin strands will be seen between the broken ends and the time is noted. By this method, the horse and cow exhibit coagulation times varying between 3-15 minutes. The other domestic species vary between 1-5 minutes.

In the tube method of Lee and White,⁶¹ 3 ml. of venous blood is drawn into a clean dry all-glass syringe. It is important that the vein be entered quickly and cleanly to avoid contamination by tissue juice and to avoid air bubbles due to excessive suction. One ml. of blood is delivered into each of three 11 mm. (I.D.) test tubes which are then placed in a 37° C. water bath if at all possible. In large animals blood may be collected directly into tubes which have previously been marked at the 1 ml. level. After 2 minutes, the first of the three tubes is gently tilted at 30-second intervals until the tube can be inverted without spillage of blood. At this time, tilting of the second tube is commenced and when this tube has clotted, the third tube is used. Timing is begun when blood first enters the syringe and the end point is reached when the blood in the third tube has clotted. Coagulation times by this method are noticeably longer than the capillary method in all the domestic animals. In the horse and cow, the normal coagulation time has been observed to range between 4 and 15 minutes, while in the dog a normal range of 3-13 minutes has been reported;⁶⁰ others⁵⁹ have recorded the mean clotting time to be for sheep 3 minutes, cow 7 minutes, cat 8 minutes and dog 8.6 minutes.

Platelet Estimations. Direct and indirect methods for the estimation

of platelet numbers have been devised. Of these, the indirect method is the more practical and will be discussed. It may be performed from the stained blood smear, which has been prepared for routine hematological examination of blood. The simplest procedure is to note the number of platelets per oil immersion field. The finding of 3 or less would suggest a thrombocytopenic condition. If the total red cell count or the white cell count is known, the number of platelets may be compared to the number of red or white cells in the smear, *e.g.*, No. of platelets/100 WBC. This relative number may be transposed into the absolute number simply by:

$$\frac{\text{No. of Platelets}}{100 \text{ WBC}} \times \text{total leukocyte count} = \text{No. of platelets/mm.}^3$$

Clot Retraction or Syneresis. Retraction of the blood clot with separation of serum is a function of the thrombocyte.⁶¹ Thus, when thrombocytopenia is suspected from results of direct observation in the stained blood film, observation of clot retraction is in order. Five ml. of blood in a clean glass tube is placed in the incubator or water bath at 37° C. The clot should be carefully loosened from the glass by means of an applicator stick after the first hour. Thereafter, the sample should be observed every hour until clot retraction begins and then at 24 hours. Failure of separation of serum within a 24-hour period indicates thrombocytopenia.

Tocantins⁶⁴ states that in normal blood of man and dog, soon after fibrin is laid down, intact thrombocytes form large knots at the intersections of fibrin needles causing the fibrin to become twisted and shortened leading to formation of a firm clot with separation of serum. He describes the thrombocytopenic clot as being heavier, softer, more jelly-like with retention of the serum in the interspaces.

BLOOD GROUPS AND CROSS MATCHING

Knowledge of blood groups in animals is of value to the veterinary practitioner because of the need from time to time for the administration of blood by transfusion. Experience in the practice of veterinary medicine has shown that, perhaps with exception of the horse,⁶⁶ there is little danger from an initial transfusion of blood to domestic animals. However, severe and even fatal reactions may develop upon administration of a second transfusion at a later date or in cases of isosensitization resulting from transplacental immunization.

Stormont⁶⁹ states that in cattle more than 60 red cell antigenic factors have been demonstrated and these are divided among 10 systems of blood groups. In sheep,⁶⁸ as in cattle, there are many antigenic factors and they fall into seven recognized systems. Swine^{65,67} have at least 16

different red cell antigens some of which have been classified in systems designated A-O, E and K. Young and colleagues⁷⁰ have demonstrated five blood factors named A (also a variant A'), B, C, D, and E in the canine. Little is known about blood groups in cats since virtually no work has been done. The blood groups of cattle are of greatest interest for parentage identification. The red cell antigenic factors of the individual animal are inherited as dominant characteristics. For this reason the various red cell antigenic factors found in an individual must be present also in its parents.

Young and colleagues⁷⁰ have found naturally occurring isoantibodies in about 15 per cent of random dogs that had never received a blood transfusion. More than half of these had an anti-D specificity, while others were either anti-B or of unknown specificity. There was no evidence that the naturally occurring isoantibodies were involved in causing transfusion reactions. However, in this connection the canine blood group A is of significance since transfusion of this blood into A-negative dogs may lead to development of anti-A which is a potent lysin both *in vitro* and *in vivo*. Antibodies for the other canine blood groups, namely, B, C, D, and E, agglutinate but do not hemolyze dog erythrocytes *in vitro*, and they appear to cause little or no damage to dog red cells *in vivo*. Young and colleagues report that 37 per cent of random dogs lacked canine A factor and 63 per cent of random donors contained A factor. From this frequency, it was calculated that approximately 25 per cent of random transfusions have the potentiality for production of anti-A in recipients. If a second transfusion is given and the donor again chosen at random, about 15 per cent of such recipients will receive incompatible blood on the second transfusion. The danger lies in administering a second or third transfusion of A-positive blood to an A-negative recipient.

Tests for Blood Groups and Blood Compatibility. The identification of the blood groups of an animal is a procedure that currently remains in the hands of a few specialists and the specific serums developed by the specialists for this purpose are not available to the veterinary practitioner. For purposes of cross-matching blood intended for transfusion, a combination test for hemolysis and erythrocyte agglutination is within the scope of the practitioner. The method is crude and negative results are not necessarily reliable. In cattle and sheep the lytic test is required since erythrocytes of these species show little or no natural ability to agglutinate. In the case of the dog and cat, the erythrocyte agglutination technic is applicable but in the horse both lytic and agglutination testing methods are required since certain equine isoantibodies act as hemolysins.

A procedure for cross-matching is as follows:

1. Blood is collected from both the donor and recipient. One sample

for red cells is taken from each in anticoagulant and one blood sample from each is allowed to clot for purposes of harvesting the serum.

2. Prepare a 5–10 per cent red cell suspension by centrifuging and washing the erythrocytes 3 times in 0.91 per cent saline.

3. Place 0.1 ml. or 2 drops of the recipient's serum and 0.1 ml. of the donor's cell suspension in a 7×60 mm. test tube and mix. In a second tube are placed equal volumes of donor's serum and recipient's cell suspension. Controls are set up in the same manner except that the donor's red cells are tested with his own serum and likewise the recipient's red cells and serum.

4. Allow all tubes to stand for 30 minutes at room temperature after which they are centrifuged for 1 minute at 500–1000 r.p.m.

5. Examine the supernatant for hemolysis. A slight hemolysis in canine blood is non-specific. Shake the tubes gently by tapping against the finger to detect grossly visible agglutination.

6. If no agglutination is observed grossly, transfer a small amount to a glass slide and examine under the low power for presence of agglutination.

Significant hemolysis and/or agglutination in either one or both of the cross-matched tubes but not in the controls indicates an incompatibility and the necessity of choosing a new donor.

EXAMINATION FOR MICROFILARIA IN CANINE BLOOD

Dirofilaria immitis, the heartworm of the dog, is an important clinical entity. The larval form, called microfilaria, occurs in the blood. *Dipetalonema* species^{73,75,77} represent another form of filariasis in dogs in the U.S.A. but their presence has not been shown to be of clinical significance. The microfilariae of *Dipetalonema* also occur in the blood while the adults are located in the subcutaneous tissues and fat. It is important to distinguish between *Dirofilaria immitis* and *Dipetalonema* sp. when microfilariae are detected on blood examination. Differentiation is based upon morphologic characteristics described by Newton and Wright.⁷⁴ Their method consists of adding 1.0 ml. of blood to 10.0 ml. of 2 per cent formalin solution, mix thoroughly, and centrifuge for 5 minutes at 1,500 r.p.m. The sediment is examined microscopically as a wet preparation after mixing with an equal volume of 1–1,000 methylene blue.

<i>Microfilaria</i> In 2% formalin	<i>Dirofilaria immitis</i>		<i>Dipetalonema</i> sp.	
	Range	Mean	Range	Mean
Length in microns	307–322	313	269–283	276
Width in microns	6.7–7.1	6.9	4.3–4.8	4.6
Tail characteristic	Mostly straight		Curved like a button hook	

Several methods for detection of the microfilarae in blood are available for routine use. These are described in the order of increasing accuracy. Where whole blood is used it may be fresh blood or blood with anti-coagulant.

Direct Blood Film. A large drop of blood is placed on a slide and covered with another slide or cover slip. The entire film is scanned under low power magnification ($100\times$). The larvae when present are viable and detected by their snake-like movement between the red cells.

Serum Method. 3 to 5 ml. of blood are allowed to clot in a test tube at room temperature. After 3 to 4 hours a drop of serum is placed under a cover slip and examined for viable larvae. The serum need not be examined immediately for viable microfilariae have been observed up to 15 days in serum of clotted blood held in the refrigerator.⁷⁶

*Whole Blood Concentration Method.*⁷² Place 1 ml. of blood into a graduated 15 ml. centrifuge tube, add 3 ml. of 3 per cent acetic acid and mix. Centrifuge at 1,500 r.p.m. for 5 minutes. Decant the supernatant and after the water adhering to the glass returns to the bottom of the tube, resuspend the sediment and make note of the volume. Remove a measured amount to a slide under a cover slip and examine under low power. Reduce the light sufficiently to give good detail. The microfilarae will be dead but can be seen quite readily as delicate filament-like structures. By counting all microfilarae under the cover slip, the count/ml. of blood can be determined by calculation from the portion of resuspended sediment examined.

Serum Concentration Method. This procedure developed by Live and Stubbs⁷¹ is said to provide about $2\frac{1}{2}$ times as many microfilarae as the whole blood concentration method. The serum which separates in 2-3 hours from 3-5 ml. of blood is added to 5 ml. of 2-5 per cent acetic acid and centrifuged at 1,500 r.p.m. for 5 minutes. The supernatant is carefully discarded and the sediment is resuspended in the few drops remaining in the test tube. The tube is inverted over a glass slide and the contents examined under low power magnification. The microfilariae are dead because of the action of the acid, as in the whole blood concentration method, but they are seen more clearly because there is much less cellular debris.

ERYTHROCYTE FRAGILITY TEST

The erythrocyte cell membrane is flexible but essentially non-elastic. This means that the cell ruptures if water is taken into the cell beyond its critical volume. The resistance of the erythrocyte to hemolysis is measured by subjecting it to decreasing concentrations of sodium chloride solution. Minimum resistance is that concentration where slight hemoly-

sis is detectable and maximum resistance is the concentration of NaCl at which hemolysis of all cells has taken place.

Marked species variations exist in erythrocyte susceptibility to hemolysis in hypotonic saline. The susceptibility is related in part to red cell size whereby increasing susceptibility correlates with decreasing red cell volume. Thus, the canine erythrocyte in health, which is about 70 cubic microns, is the most resistant and the goat erythrocyte, which is only about 20 cubic microns, is most susceptible. With decreasing size the erythrocyte approaches a spherical form thus reducing the capacity of the cell to expand without rupture.

The resistance of the erythrocyte to hemolysis may be increased or decreased in disease. This aspect has received little attention in veterinary hematology. In idiopathic hemolytic anemia it should be of interest to note whether or not the erythrocyte fragility is increased.

A method for testing erythrocyte fragility consists of exposing freshly drawn erythrocytes to hypotonic saline solutions in steps of 0.02 per cent and extending beyond both the minimum and maximum values for the species in question. The approximate normal range for each species is as follows:

<i>Species</i>	<i>Per Cent Saline</i>	
	<i>Minimum resistance</i>	<i>Maximum resistance</i>
Canine	0.50	0.32
Equine	0.56	0.39
Bovine	0.66	0.44
Feline	0.72	0.46
Ovine	0.76	0.40
Caprine	0.74	0.60

Method. Absolutely dry sodium chloride is employed to prepare a 1.0 per cent solution in a volumetric flask using distilled water. Fifteen to 18 small test tubes are placed in a single row in a rack and 1 per cent saline and distilled water are mixed to give the desired concentration of saline in a total volume of 1.0 ml. For the canine the first tube should receive 0.60 ml. saline and 0.40 ml. distilled water; the second tube 0.58 ml. saline and 0.42 ml. distilled H₂O and so on, decreasing the saline concentration by 0.02 per cent in each succeeding tube until the last tube contains 0.30 per cent saline.

Draw blood in a dry syringe and add 1 drop of the fresh blood to each tube in the rack. If blood with anticoagulant is used, centrifuge and replace the plasma with an equal volume of 0.85 per cent saline. Add 1 drop of the red cell suspension to each test tube. Allow the tubes to stand at room temperature for 2 hours and then record the saline concentrations for beginning hemolysis and complete hemolysis. A normal blood should be tested simultaneously for purposes of control.

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Chapter 3

Standardization of Nomenclature of Cells and Diseases of the Blood and Blood Forming Organs. Characterization of Immature and Mature Blood Cells

In the development of the science of hematology terminology became confused, for different investigators in some instances proposed and used the same designation for wholly different cells. Clarification and definition of terms was urgently needed for the sake of a common understanding in clinical usage and in teaching of medical students and technicians. A committee was sponsored by the American Society of Clinical Pathologists and the American Medical Association to make recommendations which, if adopted widely, would lead toward greater uniformity in usage of hematologic terms. The committee was designated as, "Committee for Clarification of the Nomenclature of Cells and Diseases of the Blood and Blood-Forming Organs." Five reports have been forthcoming,¹⁻³ also condensations and reviews of the committee's work have appeared⁴⁻⁸ and as would be expected there has been some criticism from hematologists who, though invited to work with the committee, were not able to or did not choose to participate." The Editor of *Blood*, a Journal of Hematology,⁹ commented as follows: "From the vantage point of the Editorial chair, there seems to be a good deal of merit in both points of view regarding the proposed revision and systematization of hematologic nomenclature . . . the proposed system of nomenclature, as worked out by a serious group of well-intentioned observers, is not only in the interests of simplicity, but slanted frankly for the students and the younger generation of physicians. Although some of us may dislike to have terms changed or systematized, many of the younger men in the field have evidently taken to the newer terms without too much difficulty. . . ."

Since the application of hematology in the practice of veterinary medicine is beginning to gain wider acceptance, it is proper that students of this field of medicine be taught according to the recommendations of the Committee on Nomenclature. It will be necessary to make a few modifications in order to conform to certain differences between the bloods of animals and the blood of man.

RECOMMENDED SPELLING AND DEFINITION OF TERMS

1. It is recommended that the term *leukocyte* be considered synonymous with white blood corpuscle and includes all white cells of the blood and their precursors in the blood-forming organs. This and other words derived from the same root should be spelled with a *k* and not a *c*, e.g., leukocyte, leukemia, not leucocyte or leucemia.

2. It is recommended that the descriptive terms for granules, *neutrophil*, *eosinophil*, *basophil*, and *azurophil* be spelled as indicated without a final *e*.

3. *Fine chromatin structure* is defined as having the nuclear appearance of a background of homogeneous lighter-staining parachromatin, overlaid by a darker-staining lace-net meshwork or finely stippled pattern of basichromatin, with no aggregation of the basichromatin into even a single clump of appreciable size staining darker than any other areas in the nucleus.

4. A *nucleolus* is defined as a homogeneous blue-staining area within the nucleus of a cell, which stains more like the cytoplasm than does any other part of the nucleus.

5. The term *azurophil* should be applied to the granules seen typically in the cytoplasm of cells of the lymphocytic and monocytic series and the progranulocyte stage of the granulocytic series. The term *azurophil* is recommended, and not *azure*, in describing these granules, since the term refers to an affinity for a particular dye and not to the color of the granules. These granules may be present or absent in any cell of the lymphocytic series and when present are usually coarse and in clumps (Plate VI, p. 119). They are usually present in all cells of the monocytic series including the monoblast. In the monocytic series they are usually fine, diffusely and uniformly scattered through the cytoplasm. If not seen in the monocyte or promonocyte, it usually indicates a faulty stain or poor visual definition in the microscope. These granules may be present or absent in any cell of the granulocytic series. They are rarely seen beyond the myelocyte stage except in disease. They are occasionally present in the cytoplasm of cells of the plasmacytic and erythrocytic series, and constantly present in the cells beyond the blast stage in the thrombocytic series where they tend to be fine and few in the early stages and numerous and often clumped in the more mature stages.

6. It is recognized that in each cell series there is a continuous development from the most undifferentiated to the most differentiated stage, that an indefinite number of subdivisions are possible, and that any subdivision is arbitrary. The committee recommended the use of the minimum number of subdivisions which will provide essential information for diagnostic and prognostic purposes and defined the lines of division between these stages as clearly as possible, basing these divisions on

a single easily identifiable feature. As far as possible, the feature selected to differentiate the different stages of development is one which could be recognized in either stained or supravital preparations, but it is realized that at present the majority of such decisions will be based on smears stained with Wright's stain or with one of the other Romanowsky stains. Even with these definitions, cells will be encountered where decision is difficult, in which case it is suggested that the cell be arbitrarily placed in the more differentiated category.

It is recognized that the *blast cells* of each series are morphologically very similar, all having fine nuclear chromatin structure, usually demonstrable nucleoli, and basophilic cytoplasm, with or without azurophil granules. Any cell seen in a pathological blood smear, a bone marrow preparation or a tissue imprint having the above characteristics is to be classified as a *blast cell*, to be specifically identified later by association with more mature cells of the series which accompany it. As the cell matures the chromatin becomes clumped.

7. *Disintegrated cell* is any cell of any series in which the cytoplasmic outline has been disrupted or the nuclear chromatin is no longer surrounded by a membrane, excluding the changes in the nucleus that occur in mitotic division. Disintegrated cells should be recorded as such in the differential report, even though they could be identified by dispersed granules. They should be counted even if only shreds of nuclear material are discernible, since they are undoubtedly included in the total leukocyte count.

FUNDAMENTALS OF TERMINOLOGY

Medical terms generally have Greek or Latin origin. The meaning of many commonly used terms in hematology can be readily understood by learning the following word parts:

<i>Prefix or Stem</i>		<i>Prefix or Stem</i>	
a	= without	mega	= large
aniso	= not equal	meta	= change or transformation
baso	= base	micro	= small
eosin	= red	mono	= one
erythro	= red	morpho	= form
granulo	= granule	myelo	= marrow
hem or hemat	= blood	neutro	= neutral, neither
hyper	= excessive	normo	= normal
hypo	= deficient	oligo	= scanty, few
hetero	= denoting relationship to another	pan	= all
karyo	= nucleus or nut	poikilo	= irregular
leuk	= white	poly	= many
lympho	= water, relating to lymph	pro	= before
macro	= large	reticulo	= net
		thrombo	= clot

<i>Stem or Suffix</i>	
blast	= germ
chrome	= color
crit	= to separate
cyte	= cell
meter	= to measure
ology	= study of

<i>Stem or Suffix</i>	
osis	= a process
penia	= poverty
philia	= love
plasia	= to form (tissue)
poiesis	= making
rubri	= red

Terminology for Use with Cell Variants or Diseases of the Blood.

A term not originating with the Committee on Nomenclature but included here is marked by an asterisk. Added information of veterinary interest is enclosed in parenthesis.

Anemia. A decrease in the hemoglobin value, the packed cell volume, or the erythrocyte count of more than two standard deviations below the mean normal determined by the same method on the bloods of healthy animals of the species concerned.

***Anisocytosis.** Inequality in size of cells, especially of the red blood corpuscles. (Some degree of anisocytosis may occur naturally in bovine blood.) (Plate IX, p. 247.)

***Aplastic.** Complete lack of cellular development.

Basophila and Basophilic. Terms which when applied to a cell or cells of the erythrocytic series, as observed microscopically, indicate that the cell so described shows no trace of the characteristic hemoglobin color and the cytoplasm shows a strong affinity for basophilic dyes. With the Romanowsky stains the cytoplasm of such cells stains various intensities of opaque blue.

Basophilic Stippling. A condition of the erythrocyte in which blue staining, basophilic granules are scattered throughout the cell. (Of common occurrence in the cow and sheep under conditions of intense erythropoiesis, *e.g.*, acute anaplasmosis and haemonchosis.) (Plate IX, p. 247.)

***Depression Anemia.** Refers to an anemia resulting from the selective depression of erythropoiesis, *e.g.*, chronic bacterial infection, chronic interstitial nephritis in the canine, hypothyroidism in the canine, and trichostrongylidosis in cattle and sheep.

***Diphasic Erythrocyte Sedimentation.** Failure to obtain a clear-cut demarcation between red cell mass and plasma. Suggests presence of reticulocytes and/or leptocytes that do not participate in rouleau formation and thus trail-out in the plasma zone.

Erythrocytosis. The presence of an increased erythrocyte count of more than two standard deviations above the mean normal determined by the same method on the bloods of healthy animals and *associated with an increase of total blood volume*. The latter condition distinguishes true erythrocytosis from apparent erythrocytosis associated with dehydration or hemoconcentration. (Erythrocytosis has the same connotation as polycythemia.)

Erythrocytic hypoplasia. Refers to hypoplasia of the erythrocytic series only, and means a selective depression of erythropoiesis (Depression anemia).

***Hematocrit Value.** The volume per cent erythrocytes in centrifuged blood.

***Howell Jolly Body.** Small, spherical, eccentric nuclear remnant in a young erythrocyte. Similar bodies occur normally in up to 1.0 per cent of feline and equine erythrocytes (Plate IX, p. 247).

Hypoplastic Anemia. Implies depression of all three cell types found normally in the bone marrow; namely, erythrocytic, granulocytic, and thrombocytic series. Hypoplasia is preferred to "aplasia" or "aplastic anemia" because strictly speaking, only complete absence of specific cells from the marrow should be called aplasia and this condition rarely exists in the hypoplastic anemias.

Hyperchromasia and Hyperchromatic. Terms which, when applied to the *microscopic* appearance of a cell or cells of the erythrocytic series, indicate that the cells seem to have a more intense hemoglobin color. This is never due to an increased concentration of hemoglobin but always to an increased thickness of the cell observed.

Hyperchromic. It is recommended that this term be avoided, because a significant increase in mean corpuscular hemoglobin concentration is not known to occur.

***Hypersegmenter.** A segmented neutrophil of normal size but presenting more than the normal number of nuclear lobes characteristic for the animal species concerned. The occurrence of such cells in peripheral blood indicates a need for retention of the neutrophil cell beyond its normal life span. Hypersegmentation of the nucleus is said to be the result of aging of the cell. (May be an artefact in old blood in the presence of oxalates.)

Hypochromasia and Hypochromatic. Terms which, when applied to the *microscopic* appearance of a cell or cells of the erythrocytic series, indicate that the cells described show a significant decrease in density of the characteristic hemoglobin color for the stained or unstained cell. This may be due to either thinness of the cell or decreased concentration of hemoglobin or both (Plate IX, p. 247).

Hypochromic. An adjective describing a blood picture in which the erythrocytes have a mean corpuscular hemoglobin concentration and usually a mean corpuscular hemoglobin more than two standard deviations below the mean normal determined by the same method on the bloods of healthy animals of the species concerned.

***Idiopathic.** Cause of pathologic condition is not known.

Leptocyte. A thin erythrocyte of decreased volume in relationship to its diameter, often characterized also by abnormality of shape. (**Target Cell.** A type of leptocyte commonly seen in the blood film of the canine in disease.) (Plate IX, p. 247.)

Leukemia. A neoplastic disease arising in hemopoietic tissue in which the type cells appear in the blood or are disseminated diffusely through the bone marrow.

Leukemic Leukemias: are defined as those leukemias in which the leukocyte count is above normal and the type cells are present in the blood in sufficient number to permit the diagnosis of the type of leukemia.

Subleukemic Leukemias: are those leukemias in which the leukocyte count is normal or below normal, yet the type cells of the particular variety of leukemia are present in the blood in sufficient numbers to suggest a diagnosis.

Aleukemic Leukemias: are those leukemias in which the leukocyte count is normal or below normal but the type cells either are absent or are so few, usually less than 1 per 1,000 cells, that it is impossible to make a diagnosis from examination of blood alone. In all aleukemic and most subleukemic leukemias, bone marrow examination is essential to establish a diagnosis.

***Leukemoid.** A blood picture exhibiting a marked leukocytosis with a considerable number of immature white blood cells but proven not to be true leukemia.

Macrocyte. An erythrocyte having a diameter exceeding by more than two standard deviations that of the mean normal determined by the same method on the bloods of healthy animals of the same species (Plate IX, p. 247).

Macrocytic. An adjective describing a blood picture in which the erythrocytes have a mean corpuscular volume exceeding by more than two standard deviations the mean normal determined by the same method on the bloods of healthy animals of the same species.

Macrocytosis. A preponderance of macrocytes as observed microscopically or determined by a diffraction method.

***Megaloblast.** A cell of the rubricytic series, about the prorubricyte stage, which is abnormal because of interruption of nuclear maturation. Typical of vitamin B₁₂ or folic acid deficiency.

Microcyte. An erythrocyte having a diameter more than two standard deviations below the mean normal determined by the same method on the bloods of healthy animals of the same species.

Microcytic. An adjective describing a blood picture in which the erythrocytes have a mean corpuscular volume more than two standard deviations below the mean normal determined by the same method on the bloods of healthy animals of the same species.

Microcytosis. A preponderance of microcytes as observed microscopically or determined by a diffraction method.

Myelophthistic Anemia. An anemia resulting from the displacement or crowding-out of the marrow by abnormal cells. Accurate diagnosis requires biopsy of the marrow obtained by aspiration or trephining.

Normochromasia and Normochromatic. Terms which, when applied to the microscopic appearance of a cell or cells of the erythrocytic series, indicate that the cells described appear to have their full complement of hemoglobin and no residual basophilic material in the cytoplasm; in other words, that they show normal staining characteristics.

Normochromic. An adjective describing a blood picture in which the erythrocytes have a mean corpuscular hemoglobin and a mean corpuscular hemoglobin concentration within plus or minus two standard deviations of the mean normal determined by the same method on the bloods of healthy animals of the same species.

Normocyte. Any erythrocyte having a diameter within plus or minus two standard deviations of the mean normal determined by the same method on the blood of healthy animals of the species in question.

Normocytic. An adjective describing a blood picture in which the erythrocytes have a mean corpuscular volume within plus or minus two

standard deviations of the mean normal determined by the same method on the bloods of healthy animals of the same species.

Ovalocyte. An elliptical erythrocyte. (Animals of the family Camallidae have erythrocytes that are typically elliptical: ALPACA, LLAMA, CAMAL.)

°**Poikilocyte.** An erythrocyte of irregular shape. One theory considers poikilocytes to be degradation products of erythrocytes undergoing the process of destruction. The Mexican deer, *Cervus mexicanus*, is said to have irregularly shaped erythrocytes as a normal characteristic of the animal (Plate IX, p. 247).

°**Poikilocytosis.** A blood picture showing a preponderance of poikilocytes as observed microscopically.

Pernicious Anemia Type. (Also see megaloblast.) The qualifying adjective phrase to be applied to any cell of the *erythrocytic* or *granulocytic* series, and to the marrow and blood pictures as a whole, to indicate the presence of the morphologic changes characteristically seen in pernicious anemia and other macrocytic anemias which respond to liver extract or folic acid therapy. °(Pernicious anemia does not occur in animals but this type of cell may be seen in the related macrocytic anemias in animals and it is seen in the erythrocytic series in "congenital porphyria" of the bovine.) In the nucleated cells of the erythrocytic series the major feature of this change is a relative increase in the pale-staining parachromatin with a corresponding decrease in the deep-staining basichromatin. In the cells of the granulocytic series the characteristic change is the presence of giant forms having very bizarre nuclei, and in the segmented neutrophils the occurrence of many cells with more than five lobes, hypersegmentation.

Polychromasia and Polychromatic. Terms to be applied to the microscopic appearance of a cell or cells of the erythrocytic series to indicate that they show an admixture of the characteristic colors of hemoglobin and basophilic erythrocytic cytoplasm. Young cells of erythrocytic series. Reflects intensified red cell production when polychromatophilic erythrocytes occur in numbers in peripheral blood (Plate IX, p. 247).

°**Reticulocyte.** Young erythrocyte that takes up the vital stain brilliant cresyl blue. Slightly larger than the definitive red cell and also more resistant to hemolysis. They are often polychromatophilic in the stained blood film (Plate II, p. 103).

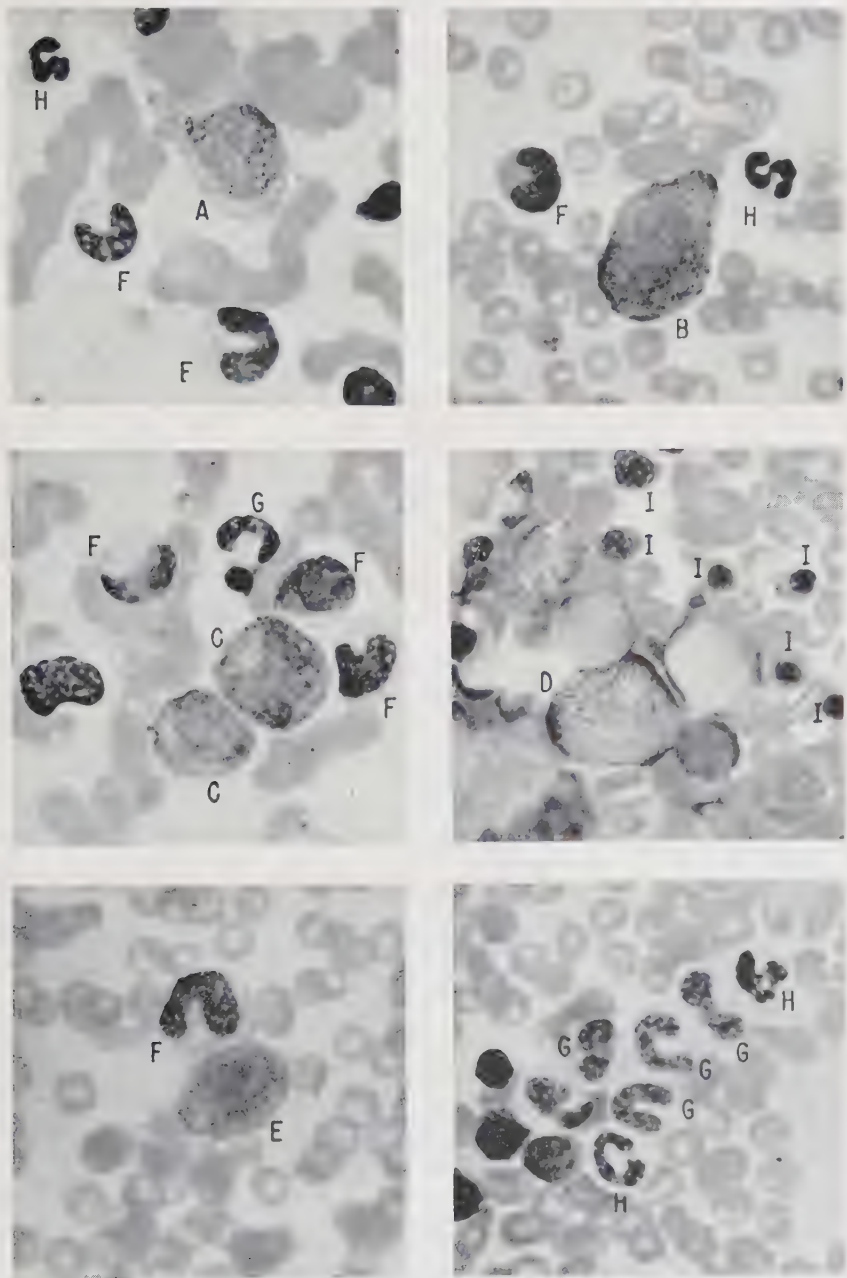
°**Rouleau (rouleaux, plural).** Erythrocytes arranged in a roll or column (Chapter 2, Fig. 9-4) suggesting a stack or roll of coins. Commonly seen in drawn blood in health in the horse and cat; may occur in the dog and pig; rare in the cow, sheep and goat.

°**Shift to the Left.** Occurrence of young forms of the granulocytic series in peripheral blood in excess of that normally encountered for the species. Generally more than 2 per cent band cells or the occurrence of younger forms is to be interpreted as a shift to the left.

Regenerative Shift to the Left: The total leukocyte count is elevated above normal and this is associated with the occurrence of immature forms of neutrophils in the peripheral blood.

Degenerative Shift to the Left: The total leukocyte count is normal

PLATE I. MATURATION OF GRANULOCYTIC LEUKOCYTES
(Aspirated Canine Bone Marrow) 600 ×



A, blast cell with nucleoli; B, progranulocyte; C, neutrophil-myelocyte; D, eosinophil-myelocyte; E, neutrophil-myelocyte with azurophil granules; F, neutrophil-metamycelocyte; G, neutrophil-band; H, mature neutrophil; I, rubricytes.

or below normal, associated with the occurrence of below normal numbers of mature neutrophils and the presence of immature neutrophils, many of which show toxic changes.

***Shift to the Right.** A blood picture characterized by an abnormal number of hypersegmented neutrophils or "aged" cells.

Spherocyte. A spheroid erythrocyte of decreased diameter in relationship to its volume and having the microscopic appearance of a hyperchromatic microcyte. (The condition may be acquired or congenital and leads to early removal of the affected erythrocytes from the circulation by the spleen.)

Toxic Neutrophils. A term which, when followed by a 1 to 4 plus designation, is recommended for the grading of toxic granules, basophilia of the cytoplasm, vacuoles and condensation of nuclear chromatin in the neutrophils. The grading should depend more on the degree of change than on the percentage of the cells involved and should be recorded in the report of the differential count.

RECOMMENDED TERMS AND DESCRIPTION OF INDIVIDUAL CELLS

<i>Name of the Series</i>	<i>Name of the Cell</i>	<i>Description</i>
Granulocytic (See PLATE I p. 97)	Myeloblast . . .	Any cell of the granulocytic series having fine chromatin structure and containing no specific granules. Usually nucleoli are visible. Cells of blast morphology found in association with progranulocytes should tentatively be classed as myeloblasts.
	Progranulocyte .	Any cell of the granulocytic series which has a nuclear structure too coarse for that of a blast cell and which has not yet developed discernible specific granules. Darkly stained azurophilic granules are often present.
	Myelocyte . . .	Any cell containing specific granules, with a round or oval nucleus. (Specific granules = neutrophilic, eosinophilic, or basophilic. The term does not include azurophilic granules.) It is distinguished from the progranulocytes by the presence of specific granules and from the metamyelocyte by the absence of indentation in the nucleus.
		This and all subsequent cells of the granulocytic series should be additionally characterized as neutrophil, eosinophil or basophil. (In the various animal species, the granules in the cells comparable to the neutrophil in man are not necessarily neutral in their staining. For this reason, the term heterophil is used by some for the neutrophil of animals.)
	Metamyelocyte .	Any cell of the granulocytic series having specific granules in the cytoplasm and a nucleus intermediate in shape between that of the myelocyte and the band cell. The nucleus usually has an indented oval shape, resembling a bean or kidney.
	Band cell . . .	Any cell of the granulocytic series which has a nucleus that could be described as a curved or coiled band. It is differentiated from the metamyelocyte by an appreciable length of the nucleus having parallel sides, and from the segmented neutrophil by having a smooth nuclear membrane showing no indentation or constriction. In veterinary hematology it is important to distinguish between the true band neutrophil and the mature monolobed neutrophil, the latter of which occurs with considerable frequency in certain animal species.

<i>Name of the Series</i>	<i>Name of the Cell</i>	<i>Description</i>
	Segmented cell	Unless this is done, the value of the differential leukocyte count as an indicator of the response to infection will be lost. Any cell containing specific granules in which the lobes of the nucleus are connected by a filament or when the nucleus is monolobed the nuclear membrane is ragged or "moth-eaten" in appearance. Since at times, in viewing a three-dimensional object from one direction, it is impossible to be certain whether two parts of the nucleus are connected by a filament or a constricted portion, it is suggested that such cells always be placed in the segmented category, since this is the more differentiated and more common cell.
Lymphocytic	Lymphoblast	Any cell of the lymphocytic series having fine chromatin structure in the nucleus. Cells of blast morphology associated with prolymphocytes should be tentatively classified as lymphoblasts.
	Prolymphocyte	Any cell of the lymphocytic series intermediate in morphology between the lymphoblast and the lymphocyte. It will always have too coarse a chromatin structure to fit the criteria for a blast and too fine a chromatin structure or too large a cell diameter to be classed as a lymphocyte.
	Lymphocyte	Any cell of the lymphocytic series having the morphology of those commonly found in the blood of healthy adults. The nucleus has a coarse chromatic structure and an eccentric position. (See discussion of term azurophil p. 91.)
Monocytic	Monoblast	Any cell of the monocytic series having fine chromatin structure. Usually nucleoli are visible.
	Promonocyte	Any cell intermediate in morphology between the monoblast and the monocyte. It is differentiated from the monoblast by having an irregularly shaped nucleus and somewhat crarser chromatin structure, and from the monocyte by the presence of one or more nucleoli.
	Monocyte	Any cell of the monocytic series having the morphology of those commonly found in the blood of healthy adults. It is differentiated from the promonocyte by the absence of nucleoli. (See discussion of term azurophil p. 91.)
Thrombocytic	Megakaryoblast	Any cell of the thrombocytic series having a nucleus with fine chromatin structure. Usually these are larger than the other blast cells.
	Promegakaryocyte	Any cell of the thrombocytic series with a nucleus containing nucleoli but having a chromatin structure too coarse for a blast cell. The nucleus is usually similar in shape to that of the megakaryocyte. Fine azurophilic granules are usually diffusely scattered through the cytoplasm.
	Megakaryocyte	Any nucleated cell of the thrombocytic series in which nucleoli are not discernible. The azurophilic granules are often aggregated into clumps. Megakaryocytes and promegakaryocytes are typically much larger than other cells found in the marrow.
	Thrombocyte	Any cell of the thrombocytic series containing no nucleus; in other words, any non-nucleated fragment of megakaryocytic cytoplasm containing azurophilic granules similar to those of the mature megakaryocyte.
	Rubriblast	A cell of the erythrocytic series having fine chromatin structure in the nucleus. Nucleoli are usually discern-

<i>Name of the Series</i>	<i>Name of the Cell</i>	<i>Description</i>
Erythrocytic (See Plate II, p, 103).		ible. A stippled chromatin pattern is more common than the lace-net pattern usually seen in other blast cells.
	Prorubricyte . . .	A cell of the erythrocytic series in which the nuclear chromatin is without pattern and the nucleus is commonly in the center of the cell and surrounded by a narrow zone of dark blue cytoplasm.
	Rubricyte . . .	Any cell of the erythrocytic series having the nuclear chromatin arranged in a pattern suggesting the spokes of a wheel (dark blobs of nuclear chromatin separated by light streaks). Some may wish to subdivide this stage further into basophilic, polychromatic or normochromatic rubricytes, according to the amount of hemoglobin present in the cytoplasm.
	Metarubricyte . .	Any nucleated cell of the erythrocytic series having pyknotic, fragmented, partially extruded or partially autolyzed nucleus. Pyknotic describes a dense, solid, structureless nuclear mass. The phenomenon of karyorrhexis or fragmentation of nuclei should be clearly distinguished from the occurrence of double, well-formed nuclei which are occasionally seen in prorubricytes and rubricytes, as well as in other cells which may divide amitotically.
	Reticulocyte . . .	A non-nucleated cell of the erythrocytic series which, when supravitality stained with brilliant cresyl blue, presents one or more granules or a diffuse network of fibrils. All reticulocytes are included under the term erythrocytes since, without a special stain, reticulocytes are indistinguishable from erythrocytes except that many reticulocytes characteristically are polychromatophilic with Wright's or Giemsa's stains.
	Erythrocyte . . .	Any non-nucleated cell of the erythrocytic series.

BONE MARROW EVALUATION

The technic for obtaining bone marrow by aspiration is described in Chapter 2, page 77. Ability to classify the various cells comes only with much study and practice, although it is possible for the veterinary student and practitioner to recognize and accurately classify the immature cells of both the granulocytic and erythrocytic series that enter peripheral blood in disease. The bone marrow is used as a source of immature cells when learning their recognition and classification.

It is helpful to study color plates of cells of bone marrow and blood when learning to classify the cells encountered in bone marrow and blood films. Many fine color atlases of human bone marrow and blood cells are available; a few that have come to the attention of the author are listed at the end of the chapter.¹⁰⁻¹⁴ Textbooks on human hematology or clinical pathology generally have one or more excellent color plates depicting the maturation sequence of the various types of blood cells. In the veterinary field, unfortunately there is a dearth of helpful material. Schermer's¹⁵ book on the blood morphology of laboratory animals has excellent color drawings of both immature and mature cells for the

laboratory animals described, included are the dog, cat, sheep and chicken. The literature contains some articles on bone marrow cells of the various domestic animals with descriptions and in some instances black and white photographs.¹⁶⁻²³

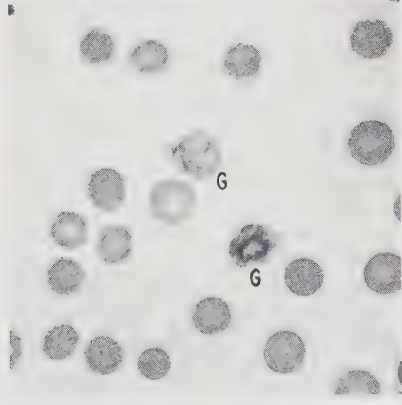
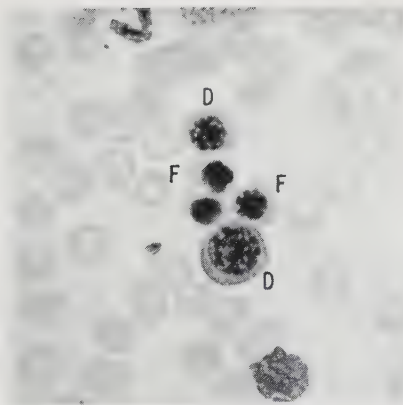
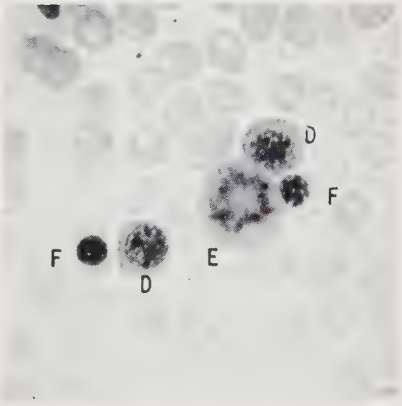
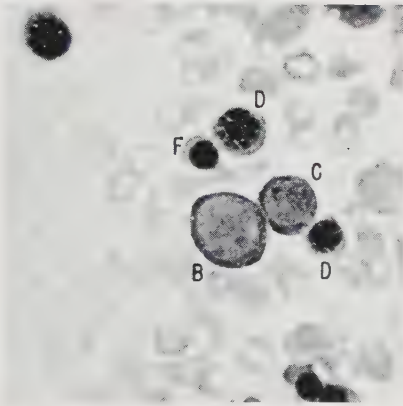
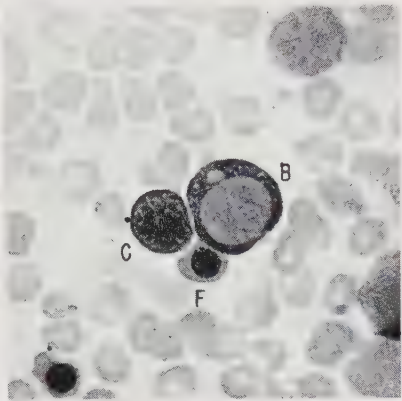
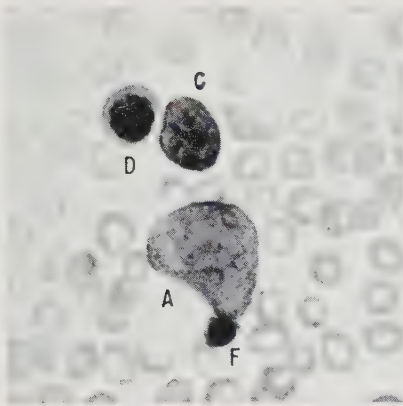
The cell descriptions prepared by the Committee on Nomenclature are slightly modified to suit characteristics in animals and should be referred to for specific characteristics of stage in the maturation series. In addition, the following general principles should be kept in mind.

- (1) The size of the cell decreases with each successive stage in maturation. (Chapter 4, *Table 26*; also see PLATES I and II, pp. 97 and 103.)
- (2) The chromatin of the nucleus is finely dispersed in the blast and pro cells and becomes more and more condensed as the cells approach maturity.
- (3) The youngest blast cells contain nucleoli which stain light blue. As cell division takes place the nucleolus loses its blue color but may persist as a ring-like structure but finally this remnant is lost also with further cell division.
- (4) The cytoplasm is basophilic in the youngest cells but becomes progressively less blue as maturation proceeds.
- (5) Azurophilic granules occur commonly in the progranulocyte stage. These disappear as the specific granules of the myelocyte stage make their appearance. Granules are not found in blast cells.
- (6) The nucleus and cytoplasm usually mature in parallel. When the maturation sequence of either cytoplasm or nucleus is out-of-step with the other, this generally indicates a disease process.
- (7) The cells of the granulocytic series are more susceptible to rupture during preparation of the film than the cells of the erythrocytic series. Thus, degenerated cells are usually of the granulocytic series.
- (8) A few lymphocytes and an occasional monocyte may appear in bone marrow aspirations. Their source is considered to be peripheral blood which is included in the aspirated marrow. Others hold that lymphocytes are more concentrated in bone marrow than in blood and that they have some function in bone marrow.

Blasts are so closely associated with the stem cells of the tissue that they are seldom removed by aspiration unless, however, the animal has displacement of the normal marrow pattern by neoplastic cells.

The progranulocyte is a heteroplastic cell capable of developing into a neutrophil, eosinophil or basophil. The myelocyte is homoplastic

PLATE II. MATURATION OF THE ERYTHROCYTIC CELLS
(Aspirated Canine Bone Marrow) 600 ×



A, blast cell with nucleoli; B, prorubricyte; C, basophilic rubricyte; D, polychromatic rubricyte; E, polychromatic rubricyte in mitosis; F, metarubricyte; G, reticulocyte.

being able to divide but limited to producing its own specific type of granulocyte. Further cell division is lost with maturation to the metamyelocyte.

BONE MARROW EVALUATION TALLY SHEET

Case No. _____ Animal _____ Date _____

<i>Cell Type</i>	<i>Total</i>	<i>Cell Type</i>	<i>Total</i>
Rubriblast		Myeloblast	
Prorubricyte		Progranulocyte	
RUBRICYTES		MYELOCYTES	
Basophilic		Neutrophilic	
Polychromatic		Eosinophilic	
Normochromatic		Basophilic	
Metarubricyte			
TOTAL CELLS ERYTHROCYTIC =		METAMYELOCYTE	
		Neutrophilic	
*Hematogones		Eosinophilic	
Lymphocytes		BAND	
Monocytes		Neutrophilic	
Unclassified cells		Eosinophilic	
		SEGMENTER	
Degenerated cells		Neutrophilic	
		Eosinophilic	
		Basophilic	
M:E =		TOTAL CELLS GRANULOCYTIC =	

*Hematogones = extruded or free nuclei of rubricytic series.

The rubricyte is the stage in which hemoglobin is synthesized and deposited in the cytoplasm. The stage may be divided into three substages based on the amount of hemoglobin, *i.e.*, basophilic, polychromatophilic, or normochromic rubricytes. Rubricytes are capable of homoplastic division but as the nucleus becomes condensed and pyknotic, cell division is no longer possible and maturation is said to have reached the metarubricyte stage.

The band cell and the reticulocyte are stages immediately before the mature definitive stage of the respective cell series. They are classified as immature cells and their occurrence in increased numbers in peripheral blood indicates intensification of bone marrow activity.

Table 5. Representative Differential Bone Marrow Counts* in Aspirated Material from Iliac Crest of the Canine in Normal and Disease States

<i>Clinical Condition</i>	<i>Normal</i>	<i>Iron Deficiency anemia in remission. (Blood + iron therapy)</i>	<i>Chronic ulcerative colitis (Blood loss)</i>	<i>Chronic interstitial nephritis</i>	<i>Lymphocytic leukemic</i>
Total Blood leukocytes	12,000	9,100	19,850	13,500	58,000
Blood PCV	45	29	15.5	22.5	21.3
Myeloblast	0.0	0.4	1.7	0.2	0.7
Progranulocyte	1.3	0.5	0.3	1.0	3.8
Myelocyte Neut.	9.0	1.1	3.3	0.2	9.8
Myelocyte Eos.	0.0	0.1	0.3	0.2	0.0
Metamyelocyte Neut.. .	7.5	2.4	6.0	7.0	25.5
Metamyelocyte Eos. . .	2.4	0.0	1.0	0.4	0.7
Band Neutrophil	13.6	9.3	12.0	20.4	17.5
Band Eosinophil	0.9	0.3	0.0	0.0	0.2
Neutrophil	18.4	8.8	23.0	34.4	11.8
Eosinophil	0.3	0.1	0.0	0.4	0.0
Basophil	0.0	0.0	0.0	0.2	0.0
Total Myeloid	53.4	23.0	47.6	64.4	70.0
Rubriblast	0.2	1.3	0.0	0.2	0.0
Prorubricyte	3.9	2.7	2.3	5.2	1.1
Rubricyte	27.0	19.0	29.6	3.0	0.7
Metarubricyte	15.3	43.0	18.2	1.0	0.0
Total Erythroid	46.4	66.0	50.1	9.4	1.8
Prolymphocyte	0.0	0.0	0.0	0.0	21.6
Lymphocyte	0.2	4.1	1.3	11.6	6.6
Monocyte	0.0	0.2	1.0	10.6	0.0
Unclassified	0.0	0.7	0.0	1.4	0.0
Degenerated	0.0	6.0	0.0	2.6	0.0
Megakaryocyte	0.0	0.0	0.0	0.0	0.0
M:E ratio	1.15	0.35	0.95	6.85	38.8
M:E including degenerated cells	—	0.44	—	7.1	—

*Bone marrow differential cell count stated in per cent.

The Myeloid:Erythroid (M:E) Ratio. This means the proportion of all granulocytic cells of the marrow to nucleated erythrocytes. A bone marrow evaluation tally sheet is helpful. An example of such a tally sheet is presented. The M:E ratio is obtained by dividing the total nucleated cells of the erythrocytic series into the total of all cells of the granulocytic series including the mature segmenters. The M:E ratio must be evaluated in the light of the total leukocyte count. When the total leukocyte count is within the normal range for the species in question, the M:E ratio is a valuable tool to indicate intensification or depression of erythropoiesis. The normal ranges and means of the M:E ratios for the various domestic animals as reported in the literature are presented in Chapter 4. A brief summary is as follows:

Dog	0.75 — 2.5,	mean 1.20:1.0
Cat	?	mean 3.40:1.0
Cow	0.27 — 2.5,	mean 0.68:1.0
Sheep	0.77 — 1.68,	mean 1.10:1.0
Horse	0.94 — 3.76,	mean 1.64:1.0
Pig	?	1.77:1.0
Goat	?	0.69 $\pm$ 1.16:1.0

With a normal total leukocyte count in peripheral blood, erythropoiesis is intensified when the ratio is smaller than normal and it is depressed when the ratio is greater than normal. Examples of bone marrow differential counts in canine diseases are presented in Table 5.

DESCRIPTION OF CELLS OF PERIPHERAL BLOOD

The stained blood film is the most useful of all hematologic procedures. The film should be scanned under high dry magnification to gain an overall impression of morphology of the erythrocytes followed by examination under oil immersion for study of thrombocytes and the differential leukocyte count.

Brief descriptions of the blood cells of the dog, cat, cow, sheep, horse and pig are given below and accompanied by photographs. In addition, some of the differential characteristics of blood cell morphology among the above named domestic animals are summarized in Table 6.

CANINE BLOOD CELLS (PLATE III)

The Erythrocytes. Rouleaux are commonly present and the cells are uniform in shape, exhibit slight variations in size and present a well-defined central pallor. An occasional "target-cell" erythrocyte is common to normal canine blood. Polychromatophilic erythrocytes are frequently seen and an occasional nucleated erythrocyte or an erythrocyte with a Howell-Jolly body may be encountered; despite the somewhat common occurrence of such young forms of red cells, their presence is not considered to be normal and may reflect an inadequate nutrition.

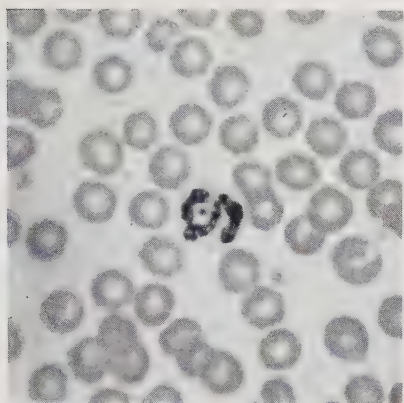
The Thrombocytes. There can be extreme variation in size, shape, color and arrangement. Usually the thrombocyte is small and presents a central cluster of purplish azurophilic granules surrounded by a pale blue matrix enclosed in a delicate membrane. Occasionally, long, thread-like processes project outward in several directions. Giant forms or amorphous masses may be present.

The Neutrophil. *Mature neutrophil.* The nucleus is irregularly lobed with rounded prominences. Filaments joining two lobes are occasionally seen but simple narrowing between lobes without true filament formation is the rule. A small nuclear sex bud is seen in some neutrophils in the female. The chromatin is clumped or plaqued into large deeply staining masses separated by lighter staining ground substance. The nuclear

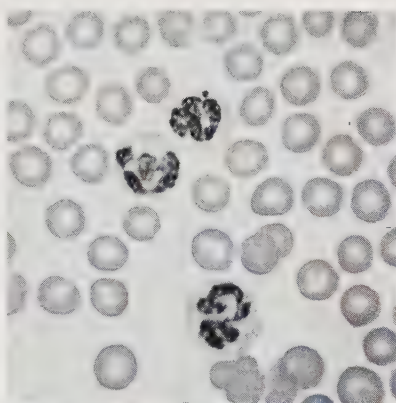
Table 6. Some Differential Characteristics in Blood Morphology of Domestic Animals

Animal	THE ERYTHROCYTES			THE LEUCOCYTES		Special Features
	Central Pallor	Mean Diameter (microns)	Reticulocytes in Peripheral Blood in Health	Approximate Neut./Lym ph. Ratio		
Dog	+	7.0	± 1.0%	70/20	Essentially uniform in size. Crenation does not occur readily.	Basophils rare. Eosinophil granules do not fill cell; granules variable in size and stain lightly. Cytoplasm shows through and takes pale blue stain.
Cat	++	5.8	± 0.5%	60/30	Crenation with few blunt processes. Slight anisocytosis. Ecentric blue body in 1 percent of cells.	Basophils rare. Eosinophil granules rod-like and stain dull grayish-orange. Majority of lymphocytes of small size. Few band neutrophils are normally present.
Cow	—	5.5	0	28/58	Anisocytosis is common. Giant forms may occur. Thrombocytes usually are numerous.	Eosinophil granules small, round, intensely stained and fill cell. Azurophil granules of large size may occur in lymphocytes. Vacuoles common in monocytes.
Sheep	±	4.5	0	30/60	Regular in size and shape with small central pale spot. Compact groups of thrombocytes.	Neutrophil nucleus usually multilobed. Eosinophil granules well-stained, ovoid and fill cell. Frequent large azurophil granules in lymphocytes. Monocyte nucleus amoeboid.
Horse	+++	5.7	0	cold-horse 55/35 hot-horse 50/45	Marked rouleaux, a consistent finding. Cells uniform in size. Occasional H-J-like body.	Eosinophil very characteristic; granules very large and fill cell. Majority of lymphocytes are small size. Monocytes usually have kidney bean nucleus.
Pig	++	6.0	± 1.0%	35/50	Crenation with sharp points is a characteristic feature. Slight anisocytosis. Occasional polychrophilia.	Eosinophil granules ovoid, pale pink-orange, fill cell. Band neutrophils occur in health. (Ave. 1 per cent.) Lymphocytes vary from small to large.

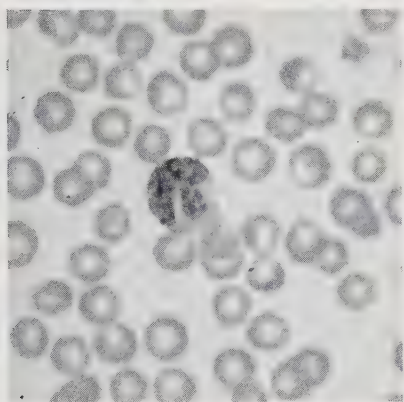
PLATE III. CANINE BLOOD CELLS (600 ×)



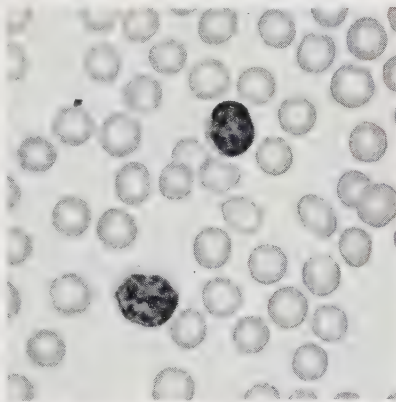
Segmented neutrophil showing a nuclear sex bud (FEMALE).



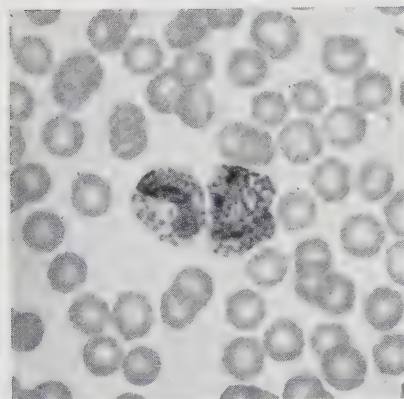
Three segmented neutrophils, one with female sex bud.



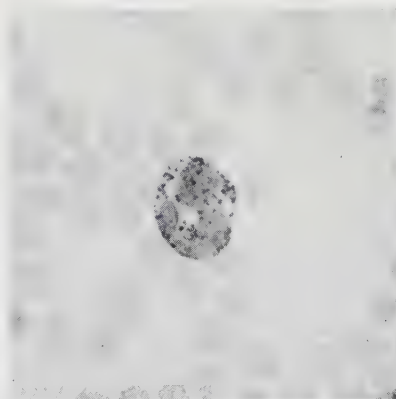
Monocyte type II.



Two lymphocytes.



Two eosinophils.



Basophil.

membrane is irregular or "moth-eaten." The cytoplasm is a faint gray-pink with fine, dust-like, diffuse granulation. Toxic granulation in the cytoplasm, as described in the human and as occurs in the bovine, is extremely rare in the canine. Toxic manifestation is usually in the form of an irregular fenestration or vacuolation of the cytoplasm.

Band neutrophil. Present in peripheral blood only in small numbers in health. The nucleus is a curved band having a smooth nuclear membrane and parallel sides for an appreciable length. The chromatin is somewhat less clumped than in the mature cell and it takes a lighter stain.

Metamyelocyte neutrophil. This cell is not found in peripheral blood in health. The nucleus varies in outline from round, with a slight indentation, to a kidney bean shape. The chromatin is more diffuse and stains less intensely than that of the mature neutrophil. The cytoplasm tends to be bluer, stains more intensely, and has a granular or ground-glass appearance. Often there is a lighter area in the region of the indentation of the nucleus and here a very pale-staining, pinkish granulation may be seen. When metamyelocytes appear in peripheral blood they are associated with a definite increase in the number of band neutrophils and this association is helpful in reaching a decision as to whether the cells in question are metamyelocytes or monocytes.

The Eosinophil. The granulation of the canine eosinophil is extremely variable. The granules may be small and regular or there may be fewer granules with considerable variation in their size and, occasionally, there may be only two or three granules of huge size, *e.g.*, 3-4 microns in diameter. The nucleus may be partially obscured by covering granules, but most commonly the nucleus is seen in its entirety for the granules are confined to the cytoplasmic area of the cell. Occasionally small vacuoles are seen which, due to their clear-cut roundness, give the impression of granules having been extruded. The cytoplasm showing through between the granules takes a light blue stain. The cell membrane tends to conform to surrounding pressures so that the cell varies in shape.

The Basophil. In both the dog and cat the basophil cell is of rare occurrence. The granules vary in number, size, and intensity of basophilic staining. The granules may be over the nucleus as well as in the cytoplasm but the maximum number is never great enough to mask the nucleus or fill the cytoplasm; the latter showing through as a gray-blue background.

The Lymphocyte. This cell varies in size but the small lymphocyte is most frequently seen. The nucleus is eccentric and the chromatin is clumped and stains deeply. The cytoplasm is pale blue with lighter staining nearest the nucleus. Dark blue azurophilic granules occur only rarely and when present they are small and few in number.

The Monocyte. Two distinct types of monocytes occur and intermediate forms can be demonstrated. Some of the noted differences can be due to the thickness of the blood film.

Type I. This cell is compact with a rounded nucleus that well fills the cell and usually shows some nuclear folding. The chromatin is lacy or almost granular and stains fairly intensely. The cytoplasm is also granular and takes a deep blue stain. Pinkish, dust-like azurophilic granulation of the cytoplasm is of common occurrence. This cell in a thick smear can be confused with the myelocyte but keeping in mind the rule that "a cell is known by the company it keeps" should be helpful for purposes of differentiation.

Type II. This monocyte is much lighter staining than is the case with Type I. The nuclear pattern is delicate and the outline is often extremely irregular with long slender processes but without filament formation. The nucleus may at times resemble that of the early band neutrophil or late metamyelocyte. However, the absence of heavy condensation of chromatin so characteristic of the neutrophilic series of cells and the presence of a basophilic cytoplasm are differential characteristics to be kept in mind. The cytoplasm is greater in proportion to the nucleus than with the type I monocyte and takes a gray-blue stain with occurrence of pinkish azurophilic granules. This monocyte is most frequently seen when the absolute monocyte count is elevated.

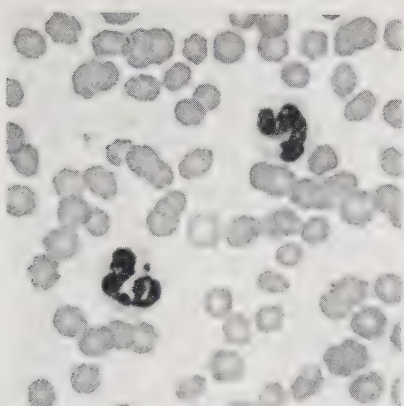
FELINE BLOOD CELLS (PLATE IV)

The Erythrocytes. Slight anisocytosis and rouleaux are characteristic. Crenation occurs often and presents itself as a few blunt processes on the cell margin. However, with marked crenation the projections of the erythrocyte cell wall are numerous and pointed. In general, the normal erythrocyte stains throughout although an ill-defined slight central pallor may be observed in some cells. A unique feature is the occurrence of Howell-Jolly-like bodies in up to 1 per cent of the erythrocytes in health.

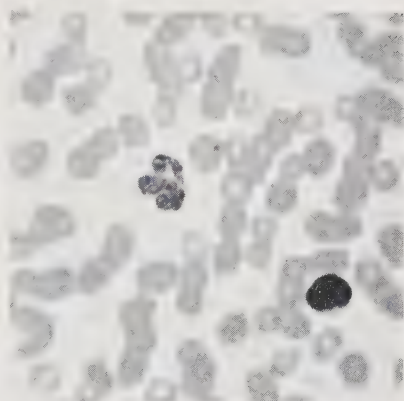
The Thrombocytes. Generally, small spherical bodies with a central cluster of purple staining azurophilic granules surrounded by a pale blue background material enclosed in a delicate membrane. The size is variable and giant forms up to the erythrocyte in size are seen. Frequently, they appear as elongated tubes and there is a tendency to formation of amorphous masses.

The Neutrophil. The nucleus well fills the cell and characteristically is a twisted coil having an irregular nuclear membrane suggesting smooth knobs. Some cells show a pinching-in of the nuclear membrane to form lobes and occasionally cells with true filaments are found. Condensation of nuclear chromatin leads to formation of darker staining plaques

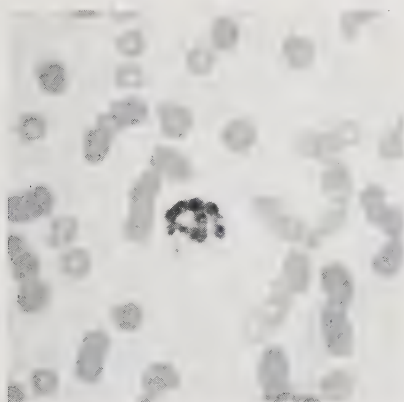
PLATE IV. FELINE BLOOD CELLS (600 ×)



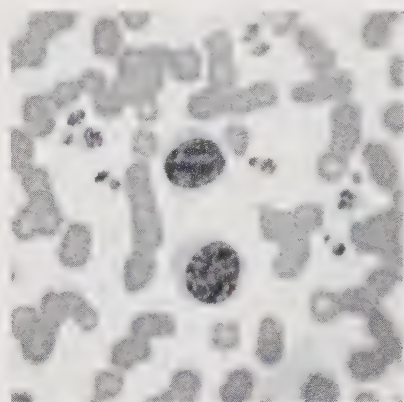
Segmented neutrophil with female sex bud and one band neutrophil.



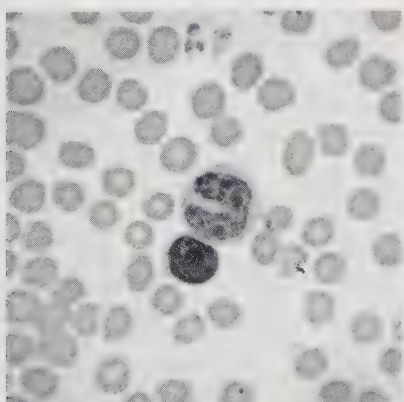
Red cell with Howell-Jolly-like body. Segmented neutrophil and small lymphocyte.



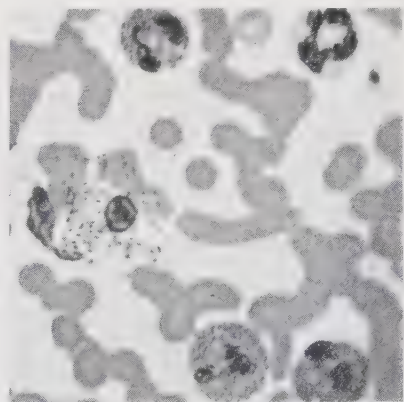
Segmented neutrophil with a few angular granules.



Two large lymphocytes and clusters of thrombocytes.



A monocyte and a small lymphocyte.



Eosinophils; one is broken showing rod-like granules. Segmented neutrophil in upper right.

separated by delicate, lighter staining lines. A small nuclear sex bud may occur in some neutrophils of the female. The cytoplasm is somewhat grayish with a delicate pink, but not prominent, granulation. Occasionally darker blue, angular granules occur singly or in numbers within the cytoplasm. The *Band Neutrophil* is seen only in small numbers in health. The cell is similar to the mature neutrophil except the nucleus is U-shaped and the nuclear outline is smooth. The *Metamyelocyte* is not seen in peripheral blood in health. The nucleus is indented like a kidney bean and the nuclear chromatin is lace-like with early condensation to form plaques. The cytoplasm is blue and granular.

The Eosinophil. The granules are rod-like, non-refractile, numerous and they may partially cover the nucleus without masking it completely. The small amount of cytoplasm that may be seen between the granules may take a very faint blue stain. The cell membrane may present an irregular contour due to the pressure of contiguous cells.

The Basophil. This cell is of such infrequent occurrence in the peripheral blood of the cat as to be almost never seen.

The Lymphocyte. The small lymphocyte is common to the cat. The nucleus is usually round but occasionally it is observed to be indented like a kidney bean. The chromatin is quite uniformly dispersed without marked condensation and the margin of the nucleus is distinct. The cytoplasm is very limited in amount and mostly to one side of the eccentrically placed nucleus. It is blue and occasionally presents a perinuclear halo with marginal condensation. Azurophilic granules are rarely seen. When observed, the granules are small, irregularly shaped, and scattered along the margin of the cytoplasm or clustered in the indentation of a kidney bean nucleus.

The Monocyte. This cell occurs only in small numbers. The nucleus is round or irregular and compact. The nuclear chromatin is lacy with some condensation; folding may be present but more often not. The cytoplasm is blue, dull, rather dark staining, slightly granular. Generally, azurophilic granulation is not seen.

BOVINE BLOOD CELLS (PLATE V)

The Erythrocytes. Anisocytosis is normal in slight degree with an occasional erythrocyte appearing to be twice the size of the smallest cells. Rouleau formation is absent in health but may become a prominent feature of the film in very sick cattle. The occurrence and degree of rouleau formation should be recorded for it may have prognostic significance. Central pallor in health is generally absent or ill-defined. In disease, however, there is a marked tendency for the erythrocytes to become bowl-shaped and the deep central concavity causes the cell center to appear to be sharply "punched-out."

The Thrombocytes. Appear as rosettes of prominent reddish-purple granules enclosed in a delicate membrane. The individual thrombocyte is usually small but giant forms up to the red cell in size may be seen. They often occur in small clusters or loose groups, or individual thrombocytes may be superimposed on erythrocytes. They may occur in large numbers and this correlates well with the size of the buffy coat of the Wintrobe hematocrit.

The Neutrophil. *Mature neutrophil.* The nuclear chromatin is well-plaquet and the nuclear membrane is often smooth but may be irregular. Short filaments between nuclear lobes are seen with relative frequency. The cytoplasm is pale pink with a dust-like granulation. Toxic granulation is seen in severe infections and is characterized by the occurrence of a few small black granules widely scattered in the cytoplasm.

Band neutrophil. The nucleus is short, fat and slightly curved. The chromatin clumps are more dispersed than in the mature cell and the cytoplasm is paler and shows less granulation.

Metamyelocyte neutrophil. The nucleus is quite thick and sometimes it is angular or bulbous but rarely kidney bean shaped. The chromatin is always plaquet. The cytoplasm tends toward pale gray and the granulation is indistinct. The cytoplasm rarely stains blue enough to lead to confusion with the monocyte.

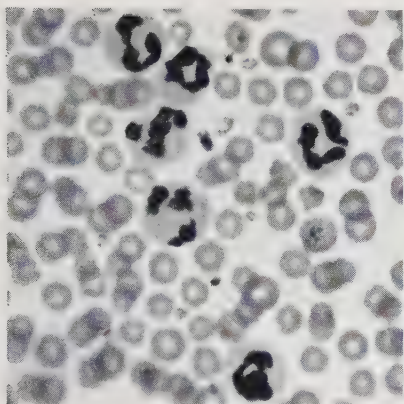
The Eosinophil. The granules are small, round, regular, jewel-like and refractile. The granules usually fill the cytoplasmic space with occasional slight bulging of the cell margin. The granules stain a true eosin color and the cytoplasm, when it can be seen, is faintly gray. The nucleus may be partially covered by the granules but is never completely masked. Band form eosinophils are common.

The Basophil. The granules are frequently tightly packed and may be scattered over the nucleus so as to partially obscure it. The granules have a purplish cast contrasting sharply with the less intensely stained nucleus. In shape the granules vary from round to elongate-oval.

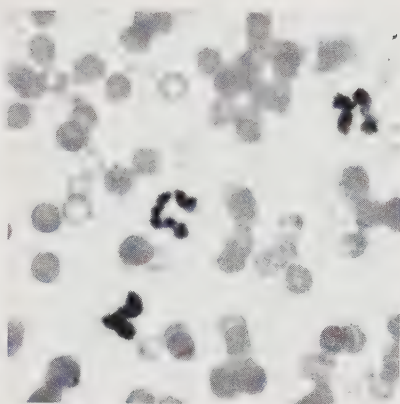
The Lymphocyte. The size varies considerably and a single film may show all gradations from small to large. The larger lymphocytes may occasionally be confused with monocytes. The *small lymphocyte* has a granular yet pyknotic compact round nucleus. The cytoplasm is small in amount and stains deep blue. The *large lymphocyte* has a much lighter staining nucleus and cytoplasm; the nucleus shows a smooth chromatin pattern and the cytoplasm may occasionally contain small vacuoles. *Azurophilic granules* are seen relatively frequently but they are not present in every bovine blood film. These granules vary considerably in size and shape; some are rod-like, others are very large and resemble blobs of nuclear material.

The Monocytes. The nucleus is variable in shape from round to convoluted. The chromatin is usually diffuse and appears stringy or

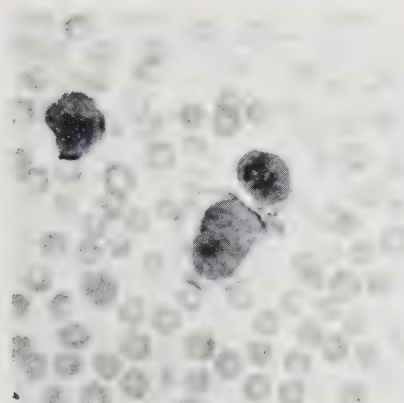
PLATE V. BOVINE BLOOD CELLS (600 ×)



Neutrophils and thrombocytes.



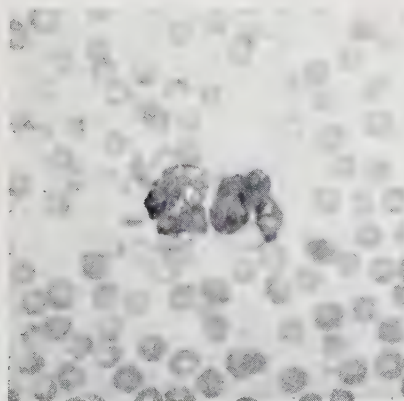
Erythrocytes in rouleaux (pathologic).



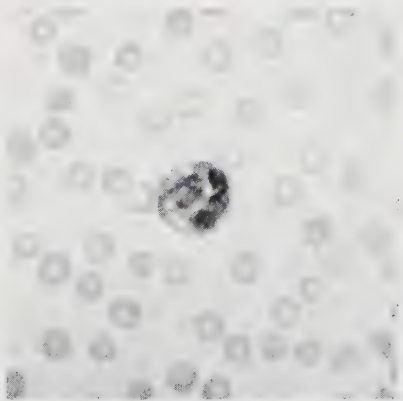
Large and small lymphocytes.



Monocytes with cytoplasmic vacuoles.

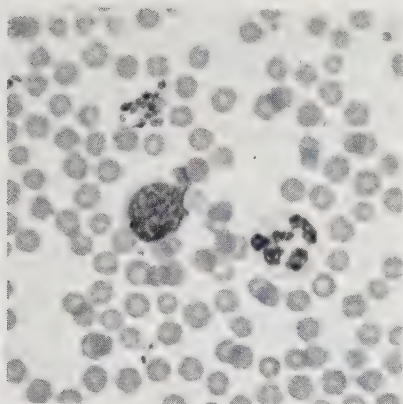


Eosinophil and Monocyte.

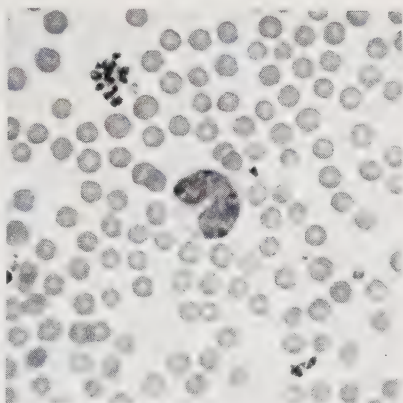


Eosinophil.

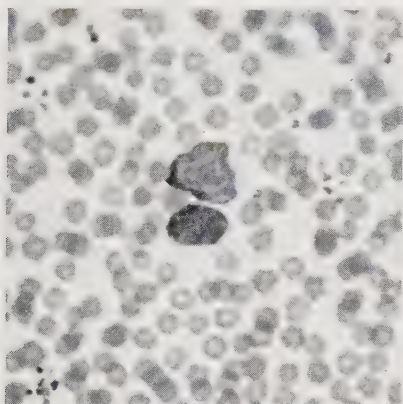
PLATE VI. OVINE BLOOD CELLS (600 $\times$)



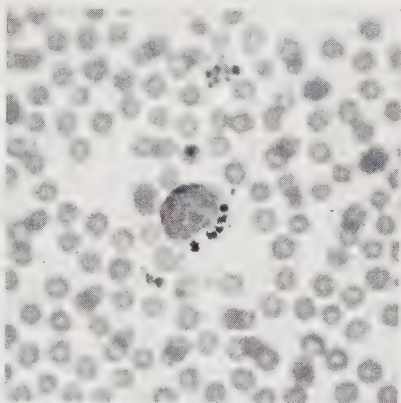
Thrombocytes, lymphocyte and neutrophil.



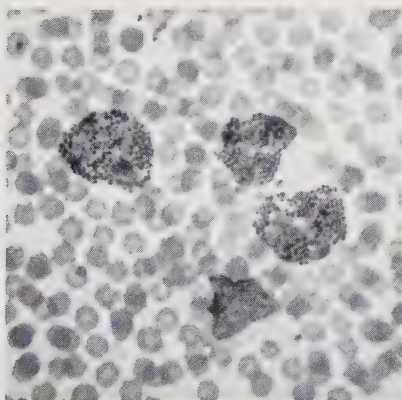
Monocyte and thrombocytes.



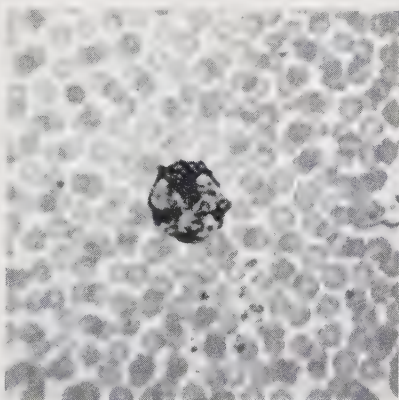
Two lymphocytes.



Lymphocyte with large azurophilic granules.



Three eosinophils and one lymphocyte.



Basophil.

granular. The cytoplasm is basophilic and granular; it is often foamy from occurrence of vacuoles. True azurophilic granules are not commonly present in the cytoplasm.

OVINE BLOOD CELLS (PLATE VI)

The Erythrocytes. There may be a slight variation in size with occasional occurrence of a giant red cell measuring at least twice normal in diameter. Short chains of rouleaux are to be anticipated in health with more marked rouleau formation in severe disease. A faint suggestion of central pallor is present and this frequently develops into a "punched-out" appearance in disease. The latter condition is produced when the erythrocyte assumes a bowl-shape with a deep central concavity.

The Thrombocytes. Small clusters of deep purple granules surrounded by a delicate membrane which is not always visible. There is a marked tendency to form compact masses which vary considerably in size. Giant thrombocytes exceeding the erythrocyte in size may be encountered. In such large forms the central cluster of azurophilic granules is surrounded by a pale blue cytoplasm enclosed in a distinct but delicate membrane.

The Neutrophil. *Mature neutrophil.* The nucleus is frequently multi-lobed, the chromatin is plaqued, filaments are short, and in the female a small nuclear sex bud may be observed in some cells. The cytoplasm stains faintly and usually slightly pinkish; the granulation is dust-like and diffuse.

Band neutrophil. The nucleus is fat and U-shaped with occasional elongation to the S form. Chromatin plaques are not as condensed as in the mature cell. The cytoplasm is slightly bluer and less granular.

The Eosinophil. The granules are uniform in size, ovoid, refractile and stain orange-red. The cytoplasm is densely packed with granules and a small number are scattered over the nucleus. A small amount of pale blue cytoplasm is sometimes apparent especially near the margin of the cell.

The Basophil. Variable number of dark granules with a reddish halo contrasting well with the lighter blue staining nucleus. The cell membrane may be irregular as if pushed into the spaces between the erythrocytes.

The Lymphocyte. Cell size is variable but the cells are not easily divided into small and large types as is the case in the bovine. The larger lymphocytes do not show characteristics that would cause them to be confused with monocytes. The nucleus of all forms has a smooth chromatin appearance and occasionally stains slightly reddish. The nuclear margin is smooth and round or oval. Infrequently, large, bizarre, binucleate forms are seen. The cytoplasm stains distinctly blue and often extends completely around the nucleus. A perinuclear halo

is commonly observed. The cytoplasm of the large lymphocytes is lighter in color and less granular. Black azurophilic granules that vary in number, size and shape may be observed occasionally. In some instances the azurophilic granules may overlay the nucleus but more commonly they are in the cytoplasm near one edge of the cell.

The Monocyte. The nucleus shows a chromatin pattern of lacy strands which stain relatively intensely. Nuclear shape is usually amoeboid, often three-lobed, with the lobes always of wide dimension. The cytoplasm is rather coarse, blue-gray and of ground glass texture. The staining of the cytoplasm is more intense than that of the large lymphocyte.

EQUINE BLOOD CELLS (PLATE VII)

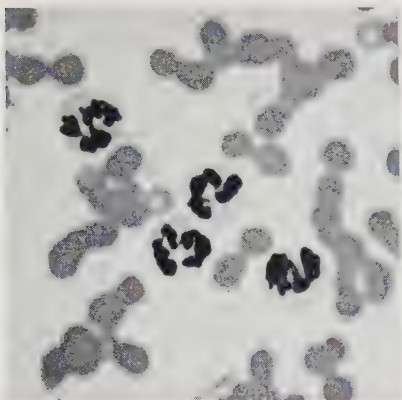
The Erythrocytes. Marked rouleau formation is the most prominent feature of the erythrocytes of the equine. Cells in rouleau stain uniformly and deeply. Individual red cells between the chains present some variation in size and slight central pallor is noted. A Howell-Jolly-like body may be found in health in a small number of erythrocytes. The body varies somewhat in size and staining but is generally black and in an eccentric position. Response of the erythropoietic tissue to blood loss appears to be unique in that reticulocytosis and polychromatophilia have not been observed to be prominent features in our cases. For a typical example see Appendix Case 13, p. 347.

The Thrombocytes. They do not stand out because staining is typically very light. The azurophilic granules are very fine and sometimes few in number. The cytoplasm takes a very light blue stain and it is enclosed in a delicate membrane. The thrombocytes vary in shape from the more common oval form to elongated sperm-like structures. Giant forms equal to the red cell in size may be observed.

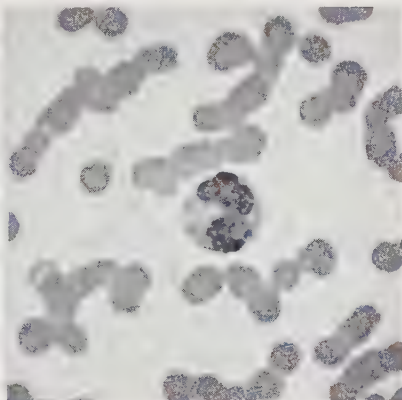
The Neutrophil. *Mature neutrophil.* The nuclear chromatin is very heavily plaqued and causes the nuclear membrane to be jagged and tends to give the impression of multilobulation. While filaments are seen at times, they are in general of rare occurrence. The cytoplasm takes a very pale stain and is filled with very fine, evenly dispersed pinkish granules.

Band neutrophil. Due to the heavy chromatin plaque formation at the nuclear margins, the nuclear outline of the equine band cell is more irregular than in band neutrophils of other species. In comparison with the mature cell, the nucleus is thicker, less knobby and usually not coiled. The cytoplasm is similar to that of the mature neutrophil. The band neutrophil may contribute 1 to 10 per cent to the differential leukocyte count in bacterial infections but the metamyelocyte neutrophil is almost never seen in peripheral blood. Neutrophilia without a marked left shift appears to be the common response to bacterial infection in the equine.

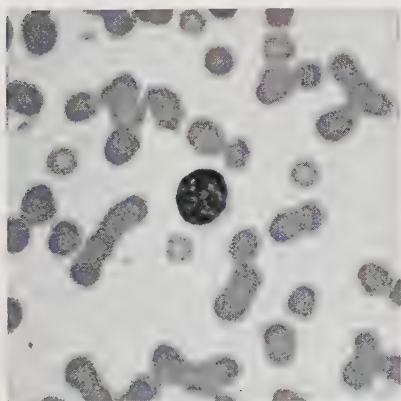
PLATE VII. EQUINE BLOOD CELLS. (600 ×)



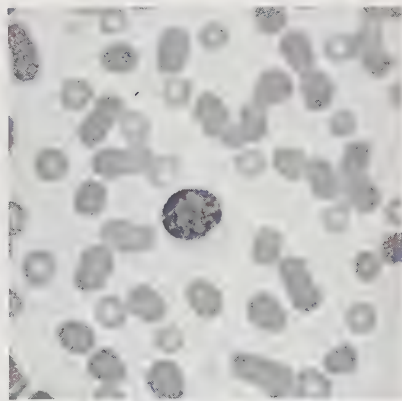
Segmented neutrophils.



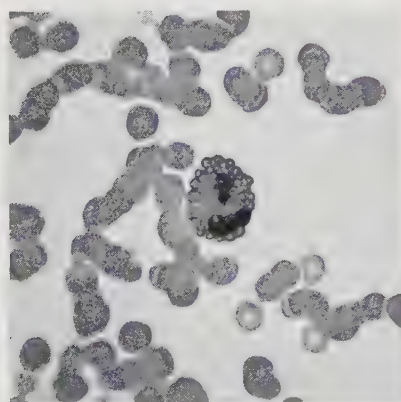
Monocyte and thrombocytes.



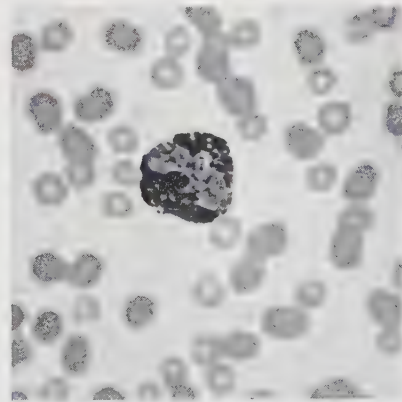
Small lymphocyte.



Large lymphocyte.



Eosinophil.



Basophil.

The Eosinophil. The granules are very large but may vary in size and they are usually tightly packed. Due to the large size of the granules the cell wall conforms to the granule contour and this gives the cell a raspberry-like appearance. The granules stain a bright orange and frequently they are so numerous as to partially obscure the nucleus. A pale blue cytoplasm may show between the granules at times and also along the edge of the cell.

The Basophil. The granules take a heavy dark stain and they are irregular in size and shape, both rod and oval forms are seen. The granules may be tightly packed or scattered irregularly in the cytoplasm with many open spaces; some may be over the nucleus and other granules may cause the cell wall to bulge.

The Lymphocyte. The majority are of small size with a heavily staining nucleus and limited cytoplasm. Lymphocytes up to the monocyte in size are encountered and these have a smooth nuclear chromatin and considerable quantity of pale blue, smooth cytoplasm. Azurophilic granules when present are few in number, small in size, irregular in shape and scattered over the cell cytoplasm without being evenly dispersed.

The Monocyte. The nucleus is most characteristically in the form of a broad kidney bean which is located to one side of the cell. Folding of the nucleus is not commonly observed. The chromatin is lacy or stringy. The cytoplasm is blue-gray, granular and pin-point pinkish azurophilic granules are scattered throughout.

PORCINE BLOOD CELLS (PLATE VIII)

The Erythrocytes. Rouleau formation is characteristic and sharp-pointed crenation is of common occurrence. Central pallor may be absent in some films and in others it is present to a variable degree. In sickness, the red cell may become bowl-shaped and the deep concavity of the bowl appears in stained films as a sharply "punched-out" central portion. Polychromasia, nucleated erythrocytes and erythrocytes with Howell-Jolly bodies are to be anticipated in the blood of piglets and growing swine up to 3 months of age.

The Thrombocytes. The predominant form is a small oval structure consisting of a cluster of deeply stained purplish granules surrounded by a pale blue cytoplasm. They are frequently gathered into irregular masses of varying size. Giant forms with finger-like extensions are occasionally seen.

The Neutrophil. *Mature neutrophil.* The nuclear chromatin is well plaqued and stains intensely. The nucleus is often coiled and the nuclear outline is very irregular but filaments commonly are not present. The

cytoplasm is a very light blue and is diffusely filled with dust-like granules that take a pink stain. The over-all color of the cytoplasm varies somewhat from pale blue to pink depending upon the intensity of staining of the granules. In oxalated blood, ingested oxalate crystals may be observed in the cytoplasm. *Band neutrophils* may make up 1 to 2 per cent of the differential leukocyte count in health. The nucleus is U- or S-shaped with a smooth nuclear membrane. Otherwise the cell is similar to the mature neutrophil. *Metamyelocyte neutrophils* may appear in peripheral blood in health during the first day post-partum. The nucleus is a fat band or kidney bean, also occasionally appears as a doughnut form, and not uncommonly as a band with enlarged or clubbed ends. The chromatin is neatly plaqued and the nuclear membrane is smooth. The cytoplasm tends toward the pale blue or basophilic side since the specific granules do not stain prominently.

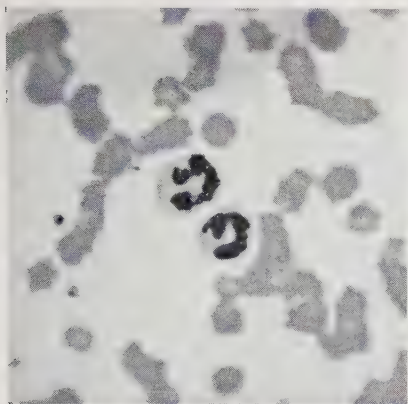
The Eosinophil. The granules are round to ovoid, stain a dull orange and completely fill the cytoplasm with a few granules also over the nucleus. The cell margin may present a cobblestone appearance due to the marked concentration of specific granules. The nucleus tends toward immaturity in that oval, kidney bean and band forms are commonly present.

The Basophil. The nucleus takes a lavender stain and the chromatin is smooth. The granules may stain similarly to the nucleus or they may take a much darker stain. They are generally limited to the cytoplasm with a few scattered over the nucleus. The shape of the granule is coccoid to dumbbell.

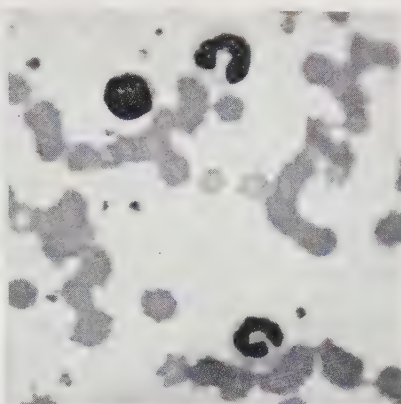
The Lymphocyte. The small lymphocyte presents a nucleus that fills the cell so that a narrow crescent of pale blue cytoplasm is all that is visible at one side or a narrow rim of cytoplasm completely surrounds the nucleus. The nucleus is oval and the chromatin is smooth but with sufficient condensation to exhibit a distinct pattern. The *large lymphocyte* has a nucleus that stains somewhat lighter and shows some plaquing of the chromatin. Occasionally ring-like structures appear in the nucleus representing nucleolar remnants but the cell is not immature in other respects. Azurophilic granules are occasionally observed in the cytoplasm of the small to medium size lymphocytes. These granules are of small size, tend to be elongated and to lie on the margin of the cell.

The Monocyte. The nucleus is irregular in outline with foldings and convolutions of its surface. The chromatin is lacy or stringy with uniformly distributed condensations. The cytoplasm is fairly voluminous and may completely surround the nucleus. It has a blue-gray, ground glass somewhat mottled appearance due to variation in intensity of staining. Azurophilic granulation is generally inconspicuous in the porcine monocyte.

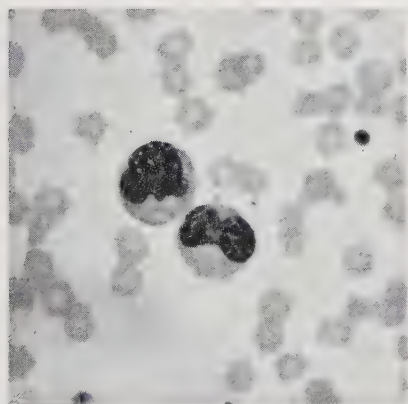
PLATE VIII. PORCINE BLOOD CELLS (600 ×)



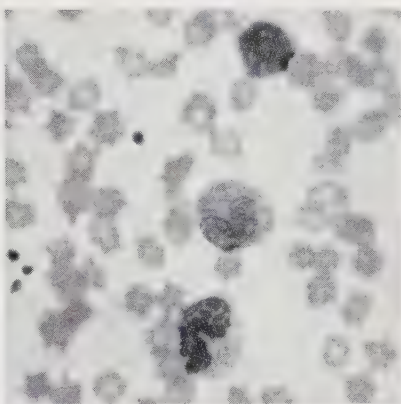
Segmented neutrophils.



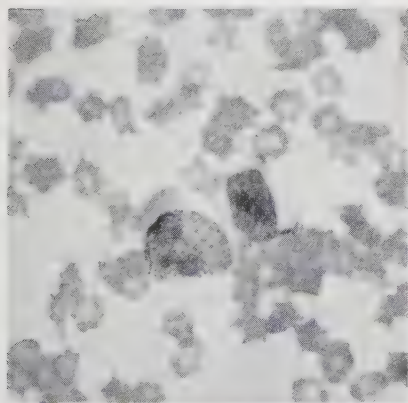
Small lymphocyte and band neutrophil at top; mature neutrophil at bottom.



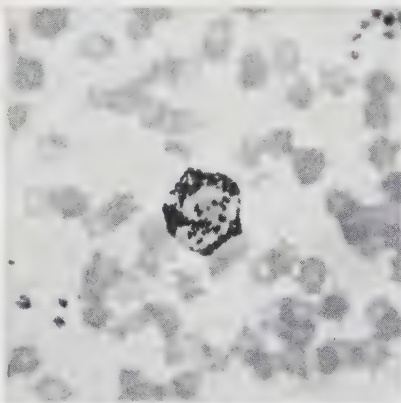
Monocytes.



Small lymphocyte (top), eosinophil (middle) and monocyte (bottom).



Eosinophil and large lymphocyte.



Basophil and thrombocytes.

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Table 7. Normal Ranges and Means for Blood Values in Domestic Animals

Animal	RBC 10 ⁶	Hb. gm. %	PCV	MCV	MCHC	WBC 10 ³	Differential Leukocyte Count in Percent				
							Band.	Neut.	Lymph.	Mono.	Eos.
Dog	5.5-8.5 (6.8)	12-18 (15)	37-55 (45)	60-77 (70)	31-34 (33)	6-18 (11)	0-3 (0.8)	60-77 (70)	12-30 (20)	3-10 (5.2)	2-10 (4)
Cat	5.5-10 (7.5)	8-14 (12)	24-45 (37)	40-55 (45)	31-35 (33)	8-25 (17)	0-3 (0.5)	35-75 (59)	20-55 (32)	1-4 (3)	2-12 (5.5)
Cow	5.0-10 (7.0)	8-14 (11)	24-48 (35)	40-60 (50)	26-34 (30)	4-12 (8)	0-2 (0.5)	15-45 (28)	45-75 (58)	2-7 (4)	2-20 (9)
Sheep	8.0-16 (12)	8-16 (12)	24-50 (38)	23-48 (32)	29-35 (32)	4-12 (9)	0-2 (0.5)	10-50 (30)	40-75 (62)	1-6 (2.5)	1-10 (4.5)
Goat	12.0-20 (15)	8-14 (11)	24-48 (35)	18-24 (20)	30-35 (32)	6-16 (12)	0-2 (0.5)	30-48 (36.5)	50-70 (55)	1-4 (2.5)	3-8 (5)
Horse (cold)	5.5-9.5 (7.5)	8-14 (11.5)	24-44 (35)	39-52 (44)	31-35 (33)	6-12 (8.5)	0-2 (0.5)	35-75 (54)	15-50 (35)	2-10 (5)	2-12 (5)
Horse (hot)	7.0-13.0 (9.8)	10-18 (13.5)	32-55 (42)	37-50 (42)	31-35 (33)	7-14 (10)	0-2 (0.5)	30-65 (49)	25-70 (44)	0.5-7 (2)	0.5-11 (4)
Pig	5.0-8.0 (6.5)	10-16 (13)	32-50 (42)	50-68 (63)	30-34 (32)	11-22 (16)	0-4 (1)	28-47 (37)	39-62 (53)	2-10 (5)	0.5-11 (3.5)

Bas.

Table 8. Normal Ranges and Means for the Absolute Differential Leukocyte Counts per cmm. of Blood

Animal	Total Leukocyte Count	Band Neutrophil	Mature Neutrophil	Lymphocyte	Monocyte	Eosinophil	Basophil
Dog	6-18,000 (11,000)	0-540 (88)	3,600-13,860 (7,700)	720-5,400 (2,200)	180-1,800 (570)	120-1,800 (440)	0 (0)
Cat	8-25,000 (17,000)	0-750 (85)	2,800-18,750 (10,000)	1,600-13,750 (5,440)	80-1,000 (510)	160-3,000 (935)	0 (0)
Cow	4-12,000 (8,000)	0-240 (45)	600-5,400 (2,240)	1,800-9,000 (4,640)	80-840 (320)	80-2,400 (720)	0-240 (40)
Sheep	4-12,000 (9,000)	0-240 (45)	400-6,000 (2,700)	1,600-9,000 (5,580)	40-720 (225)	40-1,200 (400)	0-360 (45)
Goat	6-16,000 (12,000)	0-320 (60)	1,800-7,680 (4,380)	3,000-11,200 (6,600)	60-640 (300)	180-1,280 (600)	0-320 (60)
Horse Cold	6-12,000 (8,500)	0-240 (42)	2,100-9,000 (4,590)	900-6,000 (2,975)	120-1,200 (425)	120-1,440 (425)	0-360 (42)
Horse Hot	7-14,000 (10,000)	0-280 (50)	2,100-9,100 (4,900)	1,750-9,800 (4,400)	35-980 (200)	35-1,540 (400)	0-420 (50)
Pig	11-22,000 (16,000)	0-880 (160)	3,080-10,340 (5,920)	4,290-13,640 (8,480)	220-2,200 (800)	55-2,420 (560)	0-440 (80)

Chapter 4

Normal Values in Blood Morphology

A SEARCH of the literature for normal blood values of domestic animals reveals very wide ranges and considerable variation in reported mean values, especially for the erythrocytic series. This is understandable when it is realized that the tools and methods employed in the study of blood morphology are not all precise, persons collecting and reporting data vary in training and proficiency in use of the methods, and a number of other factors may significantly influence the results; *e.g.*, nutritional level of the animals, their age, breed and degree of genetic homogeneity, and certain conditions under which the samples are drawn; such as, time of day, atmospheric temperature, fright and struggling.

In order to conserve space in the many tables of data to be found in this and other chapters, the unit of measurement or value for each blood factor is stated here and is understood to be the same in all tables of data.

<i>Blood Entity</i>	<i>Expressed in</i>
Erythrocytes	—Millions per cubic mm. of blood
Hemoglobin	—Grams per 100 ml. of blood
PCV or Packed Cell Volume	—Volume per cent
MCV or Mean Corpuscular Volume	—Cubic microns
MCH or Mean Corpuscular Hemoglobin	—Micromicrograms
MCHC or Mean Corpuscular Hemoglobin Concentration	—Volume per cent
Erythrocyte Sedimentation Rate	—mm. fall in 1 hour
Reticulocytes	—Number per 100 red cells or per cent
Nucleated Erythrocytes	—Number per 100 leukocytes
Erythrocyte Resistance to Hypotonic Saline (also called Red Cell Fragility Test)	—Per cent saline solutions producing initial and complete hemolysis
Leukocytes, total count	—Thousands per cubic mm. of blood
Leukocytes, differential count	—Per cent
Leukocytes, differential absolute count	—Number per cubic mm. of blood
Bone Marrow, differential cell count	—Per cent
Thrombocytes	—Thousands per cubic mm. of blood
Icterus Index	—Units of color as compared to standards prepared from potassium dichromate
Specific gravity	—Grams per ml.

GENERAL CONSIDERATIONS

The student will find it helpful to think of the normal blood standards as reflecting certain characteristics of the animal in question.

Hemoglobin is a respiratory pigment and its concentration in the blood, in health, is in proportion to the propensity of the animal for sustained muscular activity or ability to meet demands for sudden bursts of speed. The dog, horse and man are representative of the more active types and thus their hemoglobin needs are greater than those of the more lethargic animals, such as, the cow, sheep, goat and cat.

The size and number of erythrocytes vary among the animal species; the smaller the red cell the greater the number per unit volume of blood. The distribution of the hemoglobin in a greater number of smaller units results in increasing the surface area of the erythrocytic mass, thereby enhancing the exchange of gases. With this feature in mind, it is understandable why certain breeds of equines are best suited for use in racing and other active sports. The Arabian horse and all breeds that have stemmed from it are unique in having erythrocytes that are smaller and present in greater number than in the so-called "cold" breeds.

The goat has the smallest red cell among the domestic animals and also the greatest number, and the sheep follows as a close second. The ancestors of the sheep and goat were wont to live in the rarified atmosphere of mountain tops. In this environment, efficient respiration was required and this may in part explain why domestic sheep and goats have an arrangement for a more efficient respiration than is needed for life under domestication.

The total leukocyte number and differential distribution in peripheral blood are influenced by the secretion of the adrenal cortex, the stress hormone. The dog and cat respond well to stress and this is reflected in significant changes in their leukocyte picture in disease. On the other hand, the cow, sheep and goat respond poorly to the stress of disease insofar as their total and differential leukocyte counts are concerned. These latter animals tend to have lower total leukocyte counts in health and more lymphocytes than neutrophils.

The settling-out of red cells in their own plasma, sedimentation of erythrocytes, is dependent to a great extent upon the propensity of the erythrocyte to participate in the formation of chains or rouleaux. Rouleaux are especially prominent in drawn equine blood and, therefore, rapid sedimentation is a normal characteristic feature. The red cells of the dog, cat and pig are intermediate in tendency toward rouleau formation and, therefore, the influence of disease on the sedimentation rate can best be studied in these animals. The cow, sheep and goat red cells show little or no rouleaux and for this reason the sedimentation test has limited value in the study of the blood of these animals.

Important general references on hematology of animals are Burnett,⁴ a summarization of the literature to 1917 on the blood of the horse, cow, sheep, goat, dog, cat, pig, rabbit, and domestic fowl; Scarborough,¹⁹ an extensive compilation of data appearing in the literature through 1926 and including the rabbit, guinea pig, rat, dog, cat, cow, sheep, goat, horse, monkey, and birds; Wirth,²³ a German language text on the hematology of domestic animals; Albritton,² who, with the help of several hundred contributors and reviewers, compiled and published in 1952 a manual on Standard Values in Blood of man, the domestic animals and fowls, selected wild animals, and some of the lower vertebrates; and Schermer,²⁰ a German language text on the blood morphology of laboratory animals.

The ranges and means for the various blood values presented in this chapter and summarized in Tables 7 and 8 are a combination of data taken from the literature and original data developed in the Clinical Pathology Department of the School of Veterinary Medicine, University of California, Davis.

THE DOG

<i>Erythrocytic Series</i>			<i>Leukocytic Series</i>		
	<i>Range</i>	<i>Ave.</i>		<i>Range</i>	<i>Ave.</i>
Erythrocytes	5.5 - 8.5	6.8	Leukocytes	6 - 18,000	11,000
Hemoglobin	12.0 - 18.0	14.9	Band neut.	0 - 3	0.8
PCV	37.0 - 55.0	45.5	Neutrophil	60 - 77	70.0
MCV	60 - 77.0	69.8	Lymphocyte	12 - 30	20.0
MCH	19.5 - 24.5	22.8	Monocyte	3 - 10	5.2
MCHC	31.0 - 34.0	33.0	Eosinophil	2 - 10	4.0
Reticulocytes	0.0 - 1.5	0.4	Basophil	rare	rare
Sedimentation rate corrected to PCV value. (See Table 11)			<i>Other Data</i>		
		±5.0	Thrombocytes	2.0 - 9.0 × 10 ⁵	4.7
RBC diameter	6.7 - 7.2	7.0	Icterus Index	2 - 5	less than 5.0
Resistant to (min.)	0.40 - 0.50	0.46	Specific gravity	1.054 - 1.062	1.057
hypotonic saline (max.)	0.32 - 0.42	0.33			
M:E ratio = 0.75 - 2.5:1.0			Ave. = 1.2:1.0		

Normal blood values in the dog, for the most part, have been determined by various investigators on small groups of dogs maintained in University Departments of Biological or Medical Sciences for use in the study of physiological or medical problems. The dogs generally have been mongrels obtained from the pound and maintained under artificial conditions, that is, in cages and on laboratory diets. In one instance,³⁶ the normal values were obtained on clinically normal pets encountered in a veterinary practice. Samples of data on the erythrocytic series to be found in the literature are presented in Table 9.

Erythrocyte Number and Hemoglobin Values. At birth, the erythrocyte of the dog is very large, being over 100 cubic microns in volume,³¹ and lower in number than in the adult canine. The number of erythrocytes decreases during the first 3 weeks as the large red cells are replaced by smaller erythrocytes; thereafter, the count gradually increases until about the sixth month of life when the adult values are closely approximated. Landsberg³² and Morris³⁶ have presented mean values for dogs between 1 and 8 months of age. The levels given by Landsberg with regard to RBC and Hb are quite low and do not appear to be representative of dogs beyond the third month of life. Data presented in Table 10 were accumulated on 20 clinically normal female dogs varying from 3 months to 4 years of age and presented by their owners for spay operation. The mean hemoglobin for the 8 dogs from 3 to 6 months of age was 15 grams per 100 ml.; while for the 12 dogs from 7 months to 4 years of age, it was 16.1 grams. It should be observed that 4 of the 20 dogs had hemoglobin concentrations above 17 grams per 100 ml. Smith⁴¹ has put forth evidence that the physiologic normal hemoglobin value for the adult dog is 18 grams per 100 ml. of blood and that this level is attained in health when the diet is adequate in every respect, including an adequate supply of the B-complex vitamins.

Sex Differences. A few investigators^{28,33,34,45} have reported on the blood values separately for the sexes. In general, the conclusion has been that any apparent differences in blood values between the sexes have not been shown to be statistically significant. Andersen and Gee²⁵ observed Beagle dogs under optimum conditions of nutrition and reported higher levels for erythrocytes and hemoglobin concentration in males. These investigators also reported that during gestation the erythrocyte number became gradually reduced from a mean of 8.85 million to 4.53 million per cmm. blood at term and the total leukocyte count increased from 12,000 to 19,000 per cmm. of blood at term.

Erythrocyte Diameter. Scarborough¹⁹ gives the mean as 7.0 microns with a range in individual cells of from 6.0–8.0 microns. Richert and Brown¹⁸ state 6.9–7.3 microns, Leichsenring³⁴ found the mean size to range from 6.85–7.15 microns in 4 female dogs and from 6.8–7.21 microns in 13 male dogs, and Crandall³⁰ stated the mean for 18 dogs to be 6.7 microns.

Reticulocytes. The reticulocyte is a young erythrocyte recently released from the bone marrow. An above normal number of reticulocytes in peripheral blood is direct evidence of increased erythropoiesis in response to red cell loss or destruction. The mean number in health is in the area of 0.4–0.6 per cent of total erythrocytes,¹⁹ although Mayerson³⁵ observed the average number to be 1.85 per cent in his series of dogs, and Bruner²⁸ reported a range of 0–2.4 per cent with a mean of 0.44

Table 9. Erythrocytic Series: Compilation of Means and Ranges from the Literature for "so-called" Normal Dogs

<i>Author and Literature Reference</i>	<i>No. of Dogs</i>	<i>Sex</i>	<i>Age</i>	<i>RBC 10⁶</i>	<i>Hb. gm. %</i>	<i>PCV</i>	<i>MCV</i>	<i>MCH</i>	<i>MCHC</i>
Scarborough ¹⁹	500+	Both	Mixed	5.5-8.0 (7.2)	13.0-17.0	—	—	—	—
Powers ³⁹	25	M	Young	5.0-8.6 (7.0)	13.0-20 (15.8)	34.4-55 (45.2)	(64.6)*	(22.6)*	(35.0)*
Mayerson ³⁵	60	Both	Mixed	4.9-8.6 (6.16)	9.4-16 (13.0)	27-49 (38.6)	41.4-81.3 (59.3)	15.0-25.8 (20.0)	(33.7)*
Leichsenring ³⁴	32	Both	Adult	7.17	14.11	47.7	66.5*	19.7*	29.6*
Ashley ²⁶	43-50	Both	Adult	6.87	16.0	45.6	66.6	23.3*	34.9
Wintrobe ⁴⁵	54	Both	Mixed	7.02	14.6	47.3	67.6	21.2	31.4
Bruner ²³	34	Both	Adult	4.1-8.9 (6.45)	9.0-19 (13.56)	29-59 (44.3)	56-84 (68.9)	16-26 (21.1)	26-34 (30.7)
Landsberg ³²	95	Both	38-212 days	5.0	8.92	37.8	76.3	18.6	24.4

Morris ³⁶	35	Both	2-8 mo.	5.3	12.6	—	—	23.8*	—
	31	Both	Adult	6.2	15.1	—	—	24.3*	—
Crandall ³⁰	25	Both	Adult	6.2	12.9	44.2	71.3*	20.9*	29.2*
Mulligan ³⁷	27	?	Adult	6.76	14.4	—	—	21.3*	—
Landsberg ³³	41	M	Adult	6.28	13.8	44.4	69.3	22.6	32.4
	25	F	Adult	6.15	14.1	43.6	71.3	21.2	32.2
Van Loon ⁴⁴	81	Both	Adult	4.0-8.0 (6.2)	10.0-19.0 (14.56)	36-56 (45.6)	73.4	23.7	32.3
	18	?	1-3 day	3.61	10.5	39.8	111.1	29.4	26.4
Elderstrom ³¹	20	?	Adult	6.08	12.5	43.4	72.7	20.9	29.1
	27	?	Adult	4.8-8.1 (6.3)	10.7-20.1 (16.2)	39-60 (50)	70-91 (79)	20-32 (26)	27-37 (32)
Afonsky ²⁴	10	?	Adult	4.4-7.8 (6.1)	10.8 18.7 (14.7)	43-54 (50)	76-94 (83)	20-30 (25)	27-33 (30)
	6	?	Adult	5.2-9.0 (7.1)	13.7 22.0 (17.8)	43-57 (50)	70.4* (70.4)	17-32 (24)	29-33 (31)

* = value estimated from the data presented by the original author.

Table 10. Blood Values in 20 Clinically Normal Female Dogs Presented for Spay Operation

Age	RBC 10 ⁶	Hb. ¹ gm. %	PCV	MCV	MCH	MCHC	Retic. %	Sed. Rate mm./1 hr.	WBC 10 ³	Differential Leukocyte Count in Percentage				
										Band	Neut.	Lymph.	Mono.	Eos.
3 mo.	6.73	15.8	47.0	69.8	23.4	33.6	0.7	0	13.5	0.0	54.0	38.5	6.0	1.5
6 mo.	5.42	13.0	42.0	77.4	23.9	30.9	0.1	5	12.3	0.0	58.0	31.5	6.5	4.0
6 mo.	5.97	14.7	46.5	77.8	24.6	31.6	0.5	0	4.65	1.0	57.0	32.0	5.0	0
6 mo.	6.24	14.1	44.0	70.5	22.5	32.0	0.4	0	9.1	1.0	76.0	16.5	3.5	3.0
6 mo.	6.81	14.7	47.0	69.0	21.5	31.3	—	1	10.2	0.0	60.5	33.0	3.0	3.5
6 mo.	6.96	15.1	48.0	68.9	21.6	31.4	0.0	0	9.9	1.5	51.5	37.5	4.5	5.0
6 mo.	7.26	14.9	47.0	64.7	20.5	31.7	0.0	3	6.35	3.0	78.0	13.0	6.0	0
6 mo.	8.13	17.5	54.0	66.4	21.5	32.4	—	0	17.0	0.0	78.0	22.0	0.0	0
Mean Pups	6.69	15.0	46.9	70.6	22.4	31.9	0.28	0-3	10.37	1.0	64.0	28.0	4.3	2.7
7 mo.	6.43	16.0	49.5	76.9	24.8	32.3	0.3	0	16.05	0.5	72.5	21.0	6.0	0
8 mo.	7.20	16.1	50.0	69.4	22.3	32.2	0.2	0	12.8	0.5	76.5	19.5	3.5	0
8 mo.	5.91	13.9	45.0	76.1	23.5	30.9	0.1	0	11.9	2.0	65.5	17.0	7.5	0
9 mo.	7.04	14.9	46.0	65.3	21.1	32.4	—	3	18.3	0.5	78.0	14.0	3.0	0
1 yr.	5.37	13.2	40.0	74.4	24.5	33.0	0.8	1	8.85	1.0	74.5	15.5	7.0	0
1 yr.	6.70	15.3	47.0	70.1	22.8	32.5	0.0	0	9.25	0.0	71.5	24.0	4.5	0
1 yr.	7.11	16.9	51.0	71.7	23.7	33.1	1.4	0	10.9	0.0	66.5	27.5	0.5	0
1 yr.	7.54	16.6	54.0	71.6	22.0	30.7	0.5	1	10.3	3.0	81.5	8.5	3.0	0
1 yr.	7.56	18.1	56.0	74.0	23.9	32.3	—	0	14.5	0.0	77.0	14.0	7.0	0
1½ yr.	7.64	17.3	53.5	70.0	22.6	32.2	0.0	0 [*]	6.5	0.5	62.0	25.5	4.5	0
2 yr.	7.00	16.0	49.0	70.0	22.8	32.6	0.0	1	11.5	2.0	62.0	15.0	7.0	0
4 yr.	8.64	18.8	58.0	67.1	21.7	32.4	0.3	0	9.7	0.0	64.0	23.5	10.0	0
Mean Adult	7.01	16.1	49.9	71.4	23.0	32.2	0.36	0-5	11.7	0.5	71.0	19.0	5.3	4.2
														0

¹ Leitz photometer employing oxyhemoglobin method.

per cent. Reticulocyte counts, as shown in Table 10 on 16 dogs, were found to vary between 0 and 1.4 per cent with a mean of 0.33.

Sedimentation of Erythrocytes. The number of erythrocytes per unit volume of blood has a significant influence on their speed of settling. For this reason, it is helpful to consider the anticipated rate for each

Table 11. Relative Anticipated Erythrocyte Sedimentation Rate of Canine Blood in mm. Per 1 Hour for PCV Units from 9 to 50

PCV	Sed. rate	PCV	Sed. rate	PCV	Sed. rate
9	82	27	32	39	11
10	79	28	30	40	10
11	76	29	28	41	9
12	73	30	26	42	8
13	70	31	24	43	7
14	67	32	22	44	6
15	64	33	20	45	5
16	61	34	18	46	4
17	58	35	16	47	3
18	55	36	14	48	2
19	52	37	13	49	1
20	49	38	12	50	0
21	46				
22	43				
23	40				
24	38				
25	36				
26	34				

Record the results as follows: observed/anticipated = difference + or -.

Examples: PCV 43, Sed. rate 45: $45/7 = 38+$ severe inflammatory process.

PCV 22, Sed. rate 43: $43/43 = 0$ non-inflammatory disease.

PCV 24, Sed. rate 16: $16/38 = 22-$ abnormal red cell morphology and/or hepatopathy, or reticulocytosis. (Latter instance, there may be a diphasic sedimentation phenomenon.)

PCV unit and the difference between observed rate and anticipated rate may be attributed to the physiologic or pathologic state under consideration. Table 11 presents the anticipated erythrocyte sedimentation rate for canine cells in 1 hour for each PCV unit from 9 to 50. Simms,⁴⁰ using the Wintrobe method, determined the erythrocyte sedimentation rates of 20 blood samples from 15 apparently normal dogs. The animals varied in age from 8 weeks to maturity and both sexes were included. The distance of fall of the red cell column in 1 hour was 1 to 4 mm. in 19 blood samples and 12.5 mm. in one sample. The latter was blood from a dog which on a subsequent test gave a 2-mm. reading. It was thought that some abnormality may have existed, although not evident clinically, at the time the higher reading was ob-

tained. Simms found pregnancy to influence the rate of settling for with 20 different blood samples from 8 apparently healthy bitches, which had been pregnant 11 to 56 days, the sedimentation rates varied from 1 to 52 mm. with an average rate of fall in 1 hour of 15.4 mm. In these studies of blood sedimentation rates in the pregnant normal dog, no data are included on PCV values. The report²⁵ on blood values in the Beagle dog during gestation includes an observation on erythrocyte sedimentation rate; the ESR showed a definite increase above anticipated level for the PCV at the sixth week of gestation with a return to the normal anticipated level by the eighth week of gestation. Reference to Table 10 will reveal that among 20 clinically normal, non-pregnant female dogs, the sedimentation rates ranged between 0 and 5 mm. per hour.

Erythrocyte Fragility. A study of the pathogenesis of a hemolytic disease should include determination of the reaction of the erythrocytes to hypotonic saline. Based on the report of Musser and Krumbhaar,³⁸ the standards are given as beginning of initial hemolysis at 0.46 per cent saline and complete hemolysis at 0.33 per cent. In our laboratory, 34 determinations were made on 9 normal, female beagle dogs; the mean for initial hemolysis was 0.45 per cent saline with a standard deviation

Table 12. Compilation from the Literature of Leukocyte Values in the Dog

Author and Literature Reference	Differential Leukocyte Count in Percentage							
	WBC 10 ³	Band Neut.	Neut.	Lymph.	Mono.	Eos.	Bas.	
Burnett ⁴	6.0-12.0 (8.2)	—	60-76 (68)	11-29 (20)	3-10 (6)	1-10.5 (6)	rare	
Mayerson ³⁵	5.65-19.2 (11.16)	—	56-89 (74)	9-34 (20)	1-10 (4)	0-7 (2)	0-1 (0)	
Scarborough ¹⁹	11.84	—	60-75 (69)	10-30 (20)	2-12 (6.1)	2-10 (5)	0-2 (0.7)	
Morris ³⁶	2-8 mo.	12.2	6.7	55.8	33.3	0.1	3.4	—
	adults	11.5	6.6	65.3	22.0	0.6	5.4	—
Mulligan ³⁷	10.7	4.2	70.6	19.3	1.8	4.1	—	
Van Loon ⁴⁴	7.0-27.0 (13.5)	—	71.8	20.1	3.7	4.4	rare	
Busch (cited by Van Loon ⁴⁴)	9.3	—	65.7	21.0	6.8	5.3	—	
Musser (cited by Van Loon ⁴⁴)	15.9	—	66.7	22.1	6.8	5.1	—	

of 0.022, and for complete hemolysis the mean was 0.358 with a standard deviation of 0.025.

Leukocyte Counts, Total and Differential. The total leukocyte count and differential count with ranges and/or means as reported by several investigators are summarized in Table 12, and the findings on 20 clinically normal female dogs, presented to the clinic for spay operation, are shown in Table 10.

A significant fact is that when the classification of the band neutrophil is restricted to a cell having a nucleus which presents smooth, parallel sides, then it becomes apparent that the band neutrophil is frequently not observed in peripheral blood in the canine in health and when the cell is present it does not exceed 3 per cent of the differential count. Another item to take note of is that the lymphocyte may exceed 30 per cent in dogs under 6 months of age and there is a commensurate lowering of the neutrophil percentage, in addition, the eosinophil number tends to be less in younger dogs than in adults.

The range for total leukocyte count has been given as 6–20 thousand⁵ and 8–18 thousand⁶ in two standard texts. Experience in our clinic seems to indicate that 20,000 is too high on the one hand and 8,000 not low enough on the other hand for the anticipated range in health.

The Myeloid-Erythroid Ratio. Bloom and Meyer²⁷ have discussed the bone marrow cells of the dog in detail and have prepared a table of normal values as observed in marrow aspirated from the iliac crest. Their data, presented here, are based on the differentiation of 500 cells.

<i>Type of Cell</i>	<i>Normal Range %</i>	<i>Average %</i>	
Myeloblasts	0.00– 1.60	0.58	
Myelocyte neutrophil	0.20– 7.60	3.76	
Myelocyte eosinophil	0.00– 0.80	0.26	
Band neutrophil	16.20–29.20	23.50	
Band eosinophil	0.00– 0.80	0.12	
Neutrophil	12.04–29.60	18.50	
Eosinophil	0.00– 3.00	1.56	
Basophil	0.00– 0.20	0.02	
Heterophil	0.00– 0.20	0.02	
Total Granulocytic Cells		48.32	
Megaloblast (rubriblast)	0.00– 2.80	1.02	
Erythroblast (prorubricyte)	0.80– 4.00	2.50	M:E = 1.25:1
Normoblast (rubricyte and metarubricyte)	22.80–46.60	35.18	
Total Erythrocytic Cells		38.70	
Lymphocytes	5.80–15.20	9.80	
Pathological lymphocytes	0.00– 0.20	0.04	
Monocytes	0.20– 5.20	1.20	
Monoblast	0.00– 1.00	0.14	
Plasma cells	0.00– 2.20	0.82	
Hematogone	0.00– 1.20	0.44	
Reticulo-endothelial cell	0.00– 1.60	0.84	

Range of Myeloid-Erythroid ratio in health = 0.76 to 2.53: 1.

Mulligan³⁷ studied rib marrow smears of 35 mongrel adult dogs and observed a M:E range of 0.5 to 4.0 with a mean of 1.6. Van Loon⁴⁴ compared marrow smears from the rib and femur of 8 dogs and presented the following percentage distribution of cells:

Type of Cell	Rib Marrow		Femur Marrow	
	Mean %	Range %	Mean %	Range %
Myeloblast	0.6	0.2- 1.0	0.5	0.2- 1.0
Progranulocyte	1.6	0.7- 2.8	0.8	0.2- 1.1
Myelocytes	6.0	2.7-10.0	4.6	1.7- 8.0
Metamyelocytes	3.4	1.1- 4.6	3.3	2.0- 4.3
Band Neutrophil	11.7	6.8-17.4	12.6	10.3-18.7
Mature Neutrophil	30.1	16.8-44.2	34.7	30.1-39.7
Eosinophil	2.0	0.4- 3.8	2.0	0.2- 3.3
Monocyte	0.2	0.0- 0.3	—	0.0- 0.1
Lymphocyte	0.9	0.2- 2.7	0.7	0.0- 3.4
Rubriblast	0.6	0.2- 1.2	0.3	0.0- 0.7
Basophilic rubricyte	7.8	6.4- 9.9	6.1	4.2- 8.4
Polychromatic rubricyte	16.4	11.0-26.0	16.5	13.3-19.8
Metarubricyte	17.4	8.9-25.8	16.1	7.6-21.1
Megakaryocyte	0.5	0.0- 1.4	0.2	0.0- 0.3
Unclassified	—	0.8- 1.4	—	0.4- 3.1

M:E = 0.6 to 2.4; mean slightly over 1.0:1.0

Stasney and Higgins⁴² also have reported on the differential cell counts in the bone marrow of dogs. However, their cell terminology is difficult to adjust to terms of the standard nomenclature. Albritton's² summation of the literature on bone marrow studies involving a total of 187 dogs lists red cell series as 43.6 per cent and granulocytic cells as 52.2, which gives an M:E of 1.197:1.0.

Thrombocytes. Scarborough¹⁹ lists the findings of 6 investigators, one of whom stated that thrombocytes are relatively few in the dog and five gave means of 267,000 to 432,000 per cmm. blood. Tocantins²¹ prepared a very complete report on the mammalian platelet (thrombocyte) and lists ranges and means of his own work as well as several other investigators. Tocantins reports a range of 188,000-960,000 in 53 dogs with a mean of 461,000. Mayerson³³ reported a mean of 621,000 and Landsberg,³² in young dogs, a mean of 154,000. Andersen and Gee²⁵ give means of 252,000 in immature males, 306,000 in immature females, 407,000 in mature males and 486,000 in mature females of the Beagle breed.

Icterus Index. Using potassium dichromate standards, the icterus index of the canine in health seldom exceeds 5 units. Plasma color equal to 7.5 units should be regarded with suspicion, and a reading of 10 units or greater is distinctly abnormal.

Specific Gravity. Mayerson³⁵ observed a range of 1.0420 to 1.0562 with a mean of 1.0503 in 34 dogs. Stebbins and Leake⁴³ made observations on the diurnal variations of blood specific gravity in 6 dogs; the

lowest S.G. was 1.0453 and the highest was 1.0644; and the average maximum diurnal variation was found to be 0.0044, with a range of 0.0024 to 0.0068. Blood specific gravity determinations were made in our laboratory on 9 male and 3 female mature dogs using the copper sulfate technic described by Phillips.¹⁶ A range of 1.053 to 1.062 was recorded with a mean result of 1.057 for the 12 canine bloods. Two pups, about 2 months of age, a male and female, had blood specific gravities of 1.047 and 1.049, respectively. Ederstrom and DeBoer³¹ state that blood specific gravity is 1.0468 during the first to third day of life after which it decreases rapidly for the next 3 weeks and then gradually increases to the adult level of 1.054.

THE CAT

<i>Erythrocytic Series</i>			<i>Leukocytic Series</i>		
	<i>Range</i>	<i>Ave.</i>		<i>Range</i>	<i>Ave.</i>
Erythrocytes	5.5-10.0	7.5	Leukocytes	8-25,000	17.0
Hemoglobin	8.0-14.0	12.0	Band neut.	0-3	0.5
PCV	24.0-45.0	37.0	Neutrophil	35-75	59.0
MCV	40.0-55.0	45.0	Lymphocyte	20-55	32.0
MCH	13.0-17.0	15.0	Monocyte	1-4	3.0
MCHC	31.0-35.0	33.0	Eosinophil	2-12	5.5
Reticulocytes	0.0- 1.0	0.2	Basophil	rare	rare
Sedimentation rate corrected for			<i>Other Data</i>		
PCV (See Table 11)		±10.0	Thrombocytes	3-7 x 10 ⁵	4.5
RBC diameter	5.5- 6.3	5.8	Icterus Index	2-5	
Resistance to (min.)	0.66-0.72	0.69	Specific gravity		1.054
Hypotonic (max.) saline	0.46-0.54	0.50			

M:E ratio = 3.4:1.0 (from Sawitsky and Meyer)⁵⁹

Blood values in the normal cat are subject to considerable variation due to the excitable nature of the animal. Lamson,⁵³ as early as 1915, showed that the frightening of cats by barking dogs produced a sharp rise in the erythrocyte count. The spleen serves as a storehouse of erythrocytes and this pool of cells is forced into the circulation upon contraction of that organ under the stimulus of stress.⁵¹ Field⁴⁸ observed a similar effect of excitement on the thrombocyte count in cat's blood; the average count in 7 cats was 345,000 per cmm. and after excitement for 3 minutes the average count had risen to 508,000. The total leukocyte count is elevated by muscular activity;^{9,14} this is explained on the basis of the flushing-out of leukocytes previously trapped in collapsed capillary beds; the increase in leukocyte count is proportional to the muscular activity. Emotional states of fear, apprehension and rage result in physiological leukocytosis,⁹ although an emotional stimulus, such as taking cats for a ride in an elevator, effected no significant variation

Table 13. Some Literature References on the Blood of Cats

Reference	No. of Cats	Age Sex	RBC 10 ⁶	PCV	Hb, gm. %	MCV	MCH	MCHC	WBC 10 ³	Differential Leukocyte Count in Percentage					
										Band	Neut.	Lymph.	Mono.	Eos.	Bas.
Shaw ⁶⁶	15	—	6.8-14.6	—	—	—	—	—	8.3-53.3	—	36-75 (56.3)	16-46 (29.9)	1-13 (8.3)	1-14 (5.1)	—
Busch ⁴⁷	20	—	4.8-7.6 (6.6)	—	—	—	—	—	7.2-19.0 (13.3)	—	55.5	34.4	4.9	5.2	.035
Emmons ⁷	—	—	9.0	38.5	—	43.0	—	—	—	—	—	—	—	—	—
Scarborough ¹⁹	—	—	6.5-10.0 (8.4)	—	—	—	—	—	7.2-30.0 (13.76)	—	50-65 (57.1)	20-40 (32.5)	1-15 (5.9)	2-10 (5.3)	0-0.5 (0)
Windle ⁶²	37	adult M	9.0±1.1	40.6±4.3	12.2±1.6	45.0*	13.5*	30.0*	12.4±4.2	—	—	—	—	—	—
	64	adult F	8.4±1.2	41.3±6.9	12.0±1.4	49.2*	14.3*	29.1*	10.5±2.2	—	—	—	—	—	—

Ackart ⁴⁶	10-100	—	4.6-9.7 (7.24)	28.5-47.0 (40.15)	8.7-14.5 (11.22)	41-58 (50)	12-18 (15.5)	29-34 (31.0)	5.6-28.9 (15.0)	—	35-79 (59)	11-52 (32)	0-4 (0.7)	2-31 (8)	0-0.4 (0.01)
Hammon ⁴⁹	8-63	2-5 mo.	6.99	—	6.4-8.6 (7.7)	—	—	—	5.0-48.0 (20.86)	—	56-66	22-33	(other cells 10.2-11.5)		
Landsberg ⁵⁴	25	M	7.4±0.2	41.4±1.2	10.7±0.4	56.6± 1.1	14.9± 0.4	27.2± 0.8	17.4±1.2	—	59.3	33.0	0.8	6.9	0
	27	F	7.1±0.9	39.6±1.2	10.3±0.4	56.6± 1.2	15.5± 0.5	27.0± 0.8	16.6±1.4	—					
Riser ⁵⁸	5	8 wks.	4.3-6.2 (5.21)	—	—	—	—	—	8.0-17.5 (11.75)	—	40-71 (52.4)	23-55 (34.0)	0-5 (1.9)	0-15 (6.6)	—
	20	5 mo.- 1 yr.	6.2-9.8 (7.82)	—	—	—	—	—	10.0-31.2 (15.95)	—	26-87 (54.3)	13-70 (38.7)	0-4 (1.5)	0-15 (5.4)	—
Jennings ⁵²	25	1-6 mo.	5.7-9.0 (6.9)	32.6±3.2	9.4-12.8 (10.9)	47.2	15.8*	33.4*	9.1-22.0 (13.0)	—	40-72 (56.7)	16-45 (32.9)	1-7 (4.5)	2-10 (5.2)	0-2.0 (0.68)
Sawitzky ⁵⁹	15	—	9.5-14.8 (11.96)	—	6.0-9.0 (7.22)	—	—	—	10.0-40.5 (20.3)	0.5-3.0 (1.7)	40-81 (66.7)	14-44 (24.9)	0-5 (2.3)	2-10 (4.4)	0-1.0 (0.5)

* Estimated from author's data.

Table 14. Blood Values in Clinically Normal Cats of Various Ages

Cat. No.	Age	Sex	RBC 10 ⁶	Hb. gm. %	PCV*	MCV	MCH	MCHC	Relic. mm. hr.	Sed. Rate mm. hr.	Differential Leukocyte Count in %				
											Band	Neut.	Lymph.	Mono.	Eos. D.C. U.C.
1	7 wks.	M	7.31	11.0	—	—	15.0	—	—	—	0.5	34.0	50.5	3.0	12.0 0 0
2	7 wks.	M	7.50	11.5	—	—	15.3	—	—	—	1.0	27.0	65.0	1.5	5.5 0 0
3	7 wks.	M	7.00	10.6	—	—	15.1	—	—	—	0.5	30.5	62.0	1.5	5.5 0 0
4	less than 6 mo.	M	7.87	12.8	36.5	46	16.3	35	—	7	0.0	46.0	38.0	2.0	11.0 3.0 0
5	less than 6 mo.	?	7.78	11.2	35.0	45	14.4	32	—	27	3.0	54.0	33.0	3.0	6.0 1.0 0
5	7 mo.	F(s)	7.74	10.8	34.5	46	13.9	31	0.1	23	1.0	60.0	32.0	3.5	3.5 0 0
7	7 mo.	?	8.73	14.0	40	46	16.0	35	—	10	4.0	59.0	26.5	3.5	6.0 0.5 0.5
8	7 mo.	?	7.38	12.8	41	55	17.3	32	—	7	0.0	60.5	25.5	1.0	13.0 0 0
9	?	?	9.20	12.3	38.5	42	13.4	32	0.75	15	1.5	59.5	35.0	0.5	3.5 0 0
10	?	?	7.53	11.0	35.3	47	14.6	31	0.60	19	2.0	60.5	30.5	2.5	4.5 0 0
11	1½ yrs.	F(s)	7.08	11.8	—	—	16.6	—	0.2	—	0.0	71.5	23.5	3.5	1.5 0 0
12	mature	F(L)	7.34	11.3	—	—	15.4	—	—	—	1.0	74.5	17.5	4.5	2.5 0 0
13	mature	F(L)	9.49	12.5	—	—	13.2	—	—	—	1.0	36.0	58.0	1.0	4.0 0 0

* = when the PCV is not recorded it is because the blood was obtained from capillary puncture of ear vessel.

** = no basophils observed.

(s) = blood collected under nembutal anesthesia prior to performing spay operation.

(L) = Lactating mother cat. No. 12 was nursing Nos. 1, 2 and 3 at time of this blood work.

D.C. = degenerated cells.

U.C. = unclassified cells.

= observation not made.

of total leukocyte count.¹⁴ A mild grade of physiological leukocytosis is due to an increase in the number of polymorphonuclear neutrophils, while a very high grade of leukocytosis may show a preponderance of lymphocytes.⁹

Another problem with cats is that large vessel blood is not as conveniently obtained as from other domestic animals and, for this reason, routine blood studies are often limited to capillary blood obtained by puncturing a vessel of the ear. If the blood does not flow freely but

Table 15. Some Aspects of Postnatal Changes in the Blood of the Cat (Windle⁶²)

<i>Age</i>	<i>Number</i>	<i>RBC</i>	<i>Hb</i>	<i>PCV</i>	<i>MCV*</i>	<i>MCH*</i>	<i>MCHC*</i>	<i>MCD¹</i>	<i>WBC</i>	<i>Specific Gravity</i>
0-6 hrs.	24-24	4.95	12.2	44.7	90.3	24.6	27.3	6.7	7.55	1.0527
12-48 hrs.	23-26	5.11	11.3	41.7	81.6	22.1	27.1	—	10.18	1.0482
7 days	21-21	5.19	10.9	35.7	68.8	21.0	30.5	6.7	7.83	1.0437
14 days	18-18	4.76	9.7	31.1	65.3	20.4	31.2	6.5	8.08	1.0425
21 days	19-19	4.99	9.3	31.3	62.7	18.6	29.7	6.1	8.82	1.0444
28 days	20-20	5.84	8.4	29.9	51.2	14.4	28.1	5.9	8.55	1.0468
42 days	21-20	6.75	9.0	35.4	52.4	13.3	25.4	5.9	8.42	1.0499
56 days	19-19	7.10	9.4	35.6	50.1	13.2	26.4	5.8	8.42	1.0551
70 days	22-22	7.33	9.9	—	—	13.5	—	—	9.18	1.0478
80 days	21-21	7.69	10.3	39.0	50.7	13.4	26.4	—	9.12	1.0478
90 days	21-21	8.26	10.4	43.1	52.2	12.6	24.1	—	9.01	1.0489
120 days	21-21	8.77	10.7	35.7	40.7	12.2	29.9	—	9.36	1.0503
150 days	7-7	9.27	11.4	41.5	44.7	12.3	27.7	—	11.66	1.0494
Adult male	37-35	9.02	12.2	40.6	45.0	13.5	30.0	5.7	12.4	1.0500
Adult female	64-64	8.39	12.0	41.3	49.2	14.3	29.1	5.8	10.5	1.0512

* Estimated from the author's data.

¹ = Mean corpuscular diameter in microns.

is forced-out by squeezing the tissues, the red and white cell counts will not reflect their true peripheral blood levels.

Data on the blood of cats as found in the literature are presented in Table 13. Table 14 summarizes the data on 13 clinically normal cats varying in age from 7 weeks to maturity that were either presented to the clinic for spaying or were acquired locally for experimental use. Windle, Sweet, and Whitehead⁶² have made a detailed study of the changes occurring in the blood from birth to 150 days of age with respect to red cell number and size, hemoglobin concentration, volume per cent of erythrocytes, total leukocyte count and blood specific gravity; their data are found in Table 15.

Erythrocyte Number and Hemoglobin Values. At birth, the red cell is almost twice the cubic volume of the erythrocyte of the adult and the number per cmm. of blood is several million lower. In Table 15, the MCV, MCH, and MCHC reveal the rapidity of change in size and hemoglobin content of circulating erythrocytes as the prenatal cells are replaced by cells of smaller size. During the nursing period, the MCHC

may fall due to the lack of adequate iron intake; and thus, although red cell number is gradually increasing from the third week on, the hemoglobin concentration may not keep pace. The adult level for erythrocyte number is reached by the third to fourth month of life, while the adult level for hemoglobin concentration may not be attained until the fifth or sixth month, depending upon the availability of iron in the diet. The mean number of erythrocytes in the normal adult cat is between 7.5 and 8.5 million per cmm. and the hemoglobin concentration is about 12 grams per 100 ml. of blood.

Sex Differences. Two reports indicate slightly higher red cell counts, hemoglobin levels and total leukocyte counts for males,^{54,62} although the differences were not great. Lewis⁵⁷ compared 23 males and 16 females and also reported slightly higher erythrocyte counts and hemoglobin concentration in the males, but found the total leukocyte counts to be somewhat higher in females.

Erythrocyte Diameter. Scarborough¹⁹ cites 12 investigators who gave averages of from 5.4 to 6.5 microns; the mean of all reports being 5.9 microns. Jennings⁵² gave a range of 5.7–6.3 microns with a mean of 6.0 for kittens 1 to 6 months of age. Windle⁶² found the mean size to be 5.7 microns for the red cell of 37 adult males and 5.8 microns for 64 adult females.

Reticulocytes. The average in 25 healthy kittens, 1–6 months of age, was 0.15 per cent.⁵² Krumbhaar¹³ gave a range of 0.0–0.4 per cent with an average of 0.2 per cent. Ackart *et al.*⁴⁶ found a range of 0.07–1.10 per cent with a mean of 0.32 per cent in 20 cats.

Howell-Jolly Bodies. The occurrence of a small spherical body near the periphery of the red cell is common to cat's blood (see Plate IV, p. 113). The inclusion is similar to the Howell-Jolly body and has been reported to be constantly present in from 0.2–1.0 per cent of the erythrocytes.⁴⁹ These bodies stain dark blue or black.

Sedimentation of Erythrocytes. No mention of the sedimentation test of cat's blood was found in the literature. The limited data presented in Table 14 suggest that due to the low PCV values in normal cat's blood a rather rapid sedimentation rate should be anticipated. Until more complete information becomes available, it is suggested that Table 11 be used to compare the observed sedimentation rate and that greater than ± 10 per cent be regarded as attributable to a disease process.

Erythrocyte Fragility. Reports in the literature are extremely limited. The ranges for minimum and maximum resistance were taken from Wirth²³ and the mean values were taken from Albritton.² Hammon⁴⁹ conducted fragility tests on the blood of four kittens and reported beginning hemolysis at 0.58–0.60 per cent saline and complete hemolysis at 0.46–0.48 per cent saline. Jennings⁵² reporting on the blood values of young cats, 1–6 months of age, states that partial hemolysis

is observed at 0.40 per cent saline with complete hemolysis at 0.36 per cent.

Leukocyte Counts, Total and Differential. The fact that the total leukocyte count in cats may become elevated as a result of emotional stress and/or struggling was mentioned above. The broad normal ranges for WBC in cats, as reported in the literature, may be in part a reflection of physiologic leukocytosis. It is important, when obtaining blood from cats, to make note of any unusual evidence of fright or struggling so that the total leukocyte count may be interpreted in light of such information.

Hammon and Enders⁵⁰ report that among 80 leukocyte counts on 40 cats in good health only 3 counts were below 10,000 per cmm. of blood; of these, two were 9,000 and 8,900 and one was 5,000. The average count was 17,800 and the highest, 46,400. On the other hand, Splitter, Castro, and Kanawyer⁶¹ found the leukocyte count to range from 6,200 to 12,500 with a mean of 9,150 among 28 cats varying in age from 3 months to 2 years. Data from our own limited number of normal cats (Table 14) show a range of 9.3 to 25.2 thousand with a mean of 17,300.

In the differential count of the adult cat, the neutrophil to lymphocyte ratio and eosinophil/monocyte ratio is each about 2:1, i.e., 60/30 and 6/3. A small number of band neutrophils may be anticipated in health but generally not in excess of 3 per cent of the total count. Basophils are so rare as hardly to be taken into consideration. Hammon⁴⁹ compared the differential counts to total counts in 24 kittens and showed that as the total count increased, so did also the percentage of neutrophils. Thus, with counts between 10,000–20,000, the neutrophil percentage was 56, while with counts of 30,000 or above, the percentage was 66. This is in line with the statement made previously⁹ that a mild grade of physiological leukocytosis is due to an increase in neutrophils.

The eosinophil granules are rod-like, non-refractile, and stain a dull grayish-orange. The cytoplasm of the neutrophil may present pale blue, angular and granular masses which may be similar to Döhle inclusions and were reported⁴⁶ to have been observed in 3–80 per cent of all neutrophils of 13 cats (See Plate IV, p. 113).

The Myeloid-Erythroid Ratio. Two reports^{55,59} on the cellular composition of bone marrow of normal cats are presented here; the former is representative of aspirated marrow from the iliac crest and the latter is based on histologic sections taken from the rib, vertebra, femur or humerus at necropsy or as biopsy material.

I have taken the liberty of including the disintegrated cells in the sectioned marrow among the myeloid series of cells. Experience has shown, at least with aspirated marrow, that the cells of the granulocytic series rupture much more readily than the cells of the erythrocytic series.

	Sectioned Marrow ⁵⁵ %	Aspirated Marrow ⁵⁹ %
Myeloblasts	1.3	0.82
Progranulocytes	7.6	—
Myelocytes, neutrophilic	4.6	5.22
Myelocytes, eosinophilic	—	1.10
Metamyelocytes	9.8	7.96
Band neutrophils	13.4	30.59
Neutrophils	5.3	22.52
Eosinophils	1.5	1.71
Disintegrated cells	26.3	—
Total myeloid cells	= 69.8	= 69.9
Rubriblasts	0.7	0.35
Rubricytes	6.3	1.24
Normoblasts (polychromatic and meta- rubricytes)	8.3	18.52
Total erythroid cells	= 15.3	= 20.1
Lymphocytes	7.8	9.05
Plasma cells	0.3	0.75
Reticulum cell	—	0.02
Unclassified cells	6.2	—
M:E ratio	= 4.6:1.0	3.5:1.0

Thrombocytes. Tocantins²¹ places the ratio of erythrocyte number to thrombocytes in the cat as about 20:1.0. Lawrence and Valentine⁵⁶ made 121 counts on 55 normal cats and reported a range of 200,000 to over 600,000 with an average count of 422,000 per cmm. of blood. Field⁴⁸ found a range of 164,000 to 500,000 with a mean of 345,000 among 7 normal cats and Landsberg⁵⁴ stated the mean to be 232,143 ± 10,600.

Icterus Index. Normally less than 5 units; 10 units or more would be distinctly abnormal.

Specific Gravity. Table 15 depicts the changes in specific gravity to be anticipated during the postnatal growth period. The specific gravity of the blood of the adult feline is reported to be 1.050 for the male and 1.0512 for the female⁶² or by others as 1.054^{18,52} without reference to sex.

THE COW

<i>Erythrocytic Series</i>			<i>Leukocytic Series</i>		
	<i>Extreme Range</i>	<i>Ave. Range</i>		<i>Range</i>	<i>Ave.</i>
Erythrocytes	5.0-10.0	6.5-8.0	Leukocytes	4-12,000	7-9,500
Hemoglobin	8.0-14.0	10.5-12.0	Band neut.	0- 2	0.5
PCV	24-48	34-38	Neutrophil	15-45	28.0
MCV	40-60	45-55	Lymphocyte	45-75	58.0
MCH	11-17	13-15	Monocyte	2- 7	4.0
MCHC	26-34*	28-32*	Eosinophil	2-20	9.0
Reticulocytes	0	0	Basophil	0- 2	0.5

Sedimentation rate.			<i>Other Data</i>		
1 hour	0	0	Thrombocytes	$1.0-8.0 \times 10^5$	5.0
RBC diameter	4.5-8.0	5-6	Icterus Index	5-25	5-15
Resistance to					
hypotonic (min.)	0.52-0.66	0.48-0.62	Specific gravity	1.046-1.058	1.052
saline (max.)	0.44-0.52				
M:E ratio = 0.27-2.5:1.0; mean 0.68:1.0 from Calhoun ¹⁰⁵					

* Based on PCV from Wintrobe hematocrit
Microhematocrit results give higher MCHC

The bovine holds a position of pre-eminence among the domestic animals, for the nutrition and welfare of man, in the more advanced countries of the world, have been markedly influenced by possession of highly developed breeds of dairy cattle. It is proper, therefore, that the hematology of this highly valued animal be considered in some detail. Recent investigations in Great Britain, Sweden and locally have shown that the mean values for erythrocytes and differential values for leukocytes are influenced by age of the animal and that age of the animals must be kept in mind when blood studies are employed as an aid to the diagnosis of disease. Unfortunately, observations on the possible existence of breed differences in blood values are extremely limited. Influences of estrus, pregnancy, and parturition have come in for some attention, but critical studies on the effect of nutrition on the blood values to be considered here are lacking.

Good agreement is found in the reports of many investigators relative to the total and differential leukocyte counts, the hemoglobin concentration, and the volume per cent of erythrocytes. However, great disparity occurs with regard to mean erythrocyte counts and this, in turn, causes the erythrocytic indexes to be at variance. The inherent errors in the red cell count were discussed in Chapter 2. No doubt, the personal errors of the technicians making the counts are in part responsible; but the differences in red cell number of cattle as reported from different regions of the world are of such magnitude as to suggest that breed, climate and level of nutrition may have a significant influence on red cell number and size in the bovine.

EFFECT OF AGE UPON MEAN BLOOD VALUES OF CATTLE

Tables 17 and 18 summarize data collected at Davis on purebred dairy and beef cattle in the herds of the Animal Husbandry Department of the University. The dairy cattle were bled during the winter and spring months when atmospheric temperatures were mild. The adult cows were bled in the early morning after milking. The beef breeds were bled in July when atmospheric temperature was considerably higher, and they had to be brought from pasture and confined in corrals. The

Hereford calves were particularly fractious and considerable persuasion was required to force them into the chute for bleeding. The confinement without water and high atmospheric temperature may have been responsible for the slightly higher mean hemoglobin values in the beef cattle. The distinctly higher mean total leukocyte counts in the Hereford calves is no doubt the result of the muscular activity associated with the struggling of the animals when bled. This is explained on the basis of flushing leukocytes into the circulation that had become sequestered in the capillary beds during relative inactivity.

Table 16. Erythrocytic Values in Relation to Age in the Bovine

Data by Homan. ⁸⁵							Data by Greatorex. ^{80,81}					
<i>Age</i>	<i>No.</i>	<i>RBC</i>	<i>PCV</i>	<i>Hb.</i>	<i>MCV</i>	<i>MCHC</i>	<i>No.</i>	<i>RBC</i>	<i>PCV</i>	<i>Hb.</i>	<i>MCV</i>	<i>MCHC</i>
Birth	11	9.82	42.5	12.9	44.9	30.6	6	7.4	48.5	13.4	67.3	27.3
1 week	12	9.44	35.5	10.4	39.4	30.1	5	7.5	42.4	12.4	55.6	29.4
2 weeks	7	9.96	37.0	11.0	34.7	33.9	4	7.7	43.0	11.5	55.5	27.5
1 month	7	9.00	29.4	10.1	32.6	34.3	6	8.5	46.4	12.5	56.0	27.3
2 months	7	9.60	29.1	10.3	30.8	35.9	4	8.1	40.4	11.5	50.2	29.2
4 months	5	10.62	37.1	11.7	35.4	31.2	4	7.5	41.4	11.7	56.5	28.0
6 months	5	8.04	34.8	10.6	40.8	31.8	5	8.1	40.7	13.6	53.4	30.8
8 months	6	8.43	33.6	10.8	40.1	32.8	4	7.3	35.1	9.6	49.7	27.7
10 months	7	8.14	35.7	10.3	42.1	31.3	4	6.4	34.9	11.7	52.7	33.0
12 months	7	7.65	35.9	10.4	43.5	31.6	4	7.5	36.9	9.0	50.7	25.7
18 months	6	7.20	33.1	10.9	49.3	31.8	—	—	—	—	—	—
24 months	3	6.43	35.0	11.9	54.3	34.3	—	—	—	—	—	—
30 months	2	6.40	34.5	10.4	54.0	30.0	—	—	—	—	—	—
Cows		5.97	33.6	11.3	57.0	33.6		5.7	37.4	12.0	67.1	33.7

The Red Cells in Young Bovine. Holman⁸⁵ and Greatorex^{80,81} have presented data on the changes occurring with increasing age in young cattle in Great Britain. Holman's material was limited to the Ayrshire breed, while Greatorex included four dairy breeds and one beef breed. Table 16 presents a portion of the much more extensive data from the reports of these two British investigators.

These two sets of data together with local results summarized in Table 17 reveal the marked disparity in red cell counts, and indexes derived therefrom, encountered in published reports on the hematology of cattle. Until technics are developed and widely used that are free from inherent errors and the personal equation of the technician, it appears impossible to use normal data on red cell number collected in one locality for application to clinical interpretation elsewhere.

There is general agreement, however, that red cell counts are highest in calves and decrease gradually until the adult level is reached between 18 and 36 months of age.^{19,73,75,85,97,110,112} The red cells of calves at birth,

while small and numerous, do not exhibit evidence of immaturity. Fraser¹¹⁰ found reticulocytes up to 1.4 per cent but not beyond the second day of post-natal life. Some calves even within a few hours after birth failed to show reticulocytes in peripheral blood. Fraser also stated that punctate basophilia (basophilic stippling) was observed along with reticulocytes but not beyond the forty-eighth hour. Blount⁶⁶ reported less than 1 per cent reticulocytes in calves and stated that normoblasts are very rare. He concluded that the normal bovine passes only mature normocytes into the blood stream and, therefore, the erythropoietic system shows few features illustrating the adjustment of the young calf to its extra-uterine existence. Blount called attention to the existence of anisocytosis as a normal feature of bovine blood and stated that biconcavity of the erythrocyte is not common, many cells staining throughout. Greator⁸⁰ agreed that anisocytosis may be displayed at any age but particularly in the early weeks. However, he failed to find reticulocytes or punctate basophilia while a few normoblasts were observed but they were exceedingly rare.

The PCV and Hemoglobin in Young Bovines. The volume per cent of erythrocytes and hemoglobin are relatively high at birth and become reduced as soon as the calf ingests colostrum⁹⁷ as a result of dilution of the plasma. During the nursing period, however, when iron intake is low and the calf is increasing in size, the PCV and Hb. may decline steadily over a period of weeks and even months reaching levels as low as 28 to 30 per cent for PCV and 8 to 9 grams Hb./100 ml.^{72,102} In addition to the influences of iron intake on Hb. and red cell number, the changes in erythrocyte number and size produced by increasing age will be reflected in the harmonious fall and rise of the PCV.⁸⁵

The Leukocytes in Young Bovines. Some investigators claim higher leukocyte counts for calves than adults.^{73,81,110} Holman⁸⁵ states that although the average leukocyte count in calves is similar to adults, a higher proportion of calves show physiological leukocytosis and few calves show counts under 6,000. Table 18 reveals no significant differences in mean total leukocyte counts between cattle less than 1 year old and older animals with the exception of the immature Herefords and this deviation was attributed to excitement of the animals when bled. Table 17, however, in which the data are divided into specific age groups, suggest that lower total leukocyte counts may be anticipated in aged cattle. Moberg⁹² states that the total white count and absolute number of the neutrophils, lymphocytes and monocytes decrease with age, while with the eosinophils the opposite is the case. Furthermore, the number of circulating lymphocytes is low in the newborn. This corresponds with the undeveloped state of the lymphocytic organs. Later, as antibodies are needed, the lymphocytic tissue expands and circulating lymphocytes increase. With age, and perhaps atrophy of the thymus, the circulating

Table 17. Mean Blood Values in Purebred Jersey Female Cattle as Influenced by Age

No. Animals	Age	RBC 10 ⁶	Hb. gm. %	PCV	MCV	MCH	MCHC*	WBC	Differential Leukocyte Count in Percentage			
									Neut.	Lymph.	Mono.	Eos.
6	3½-4½ months	13.10	11.07	36.16	27.6	8.45	30.6	7,567	28.2	62.9	8.0	0.8
5	7½-9 months	10.65	10.10	30.40	28.5	9.48	33.2	8,000	8.8	82.2	8.0	1.0
8	11-12 months	8.62	9.60	28.10	32.6	11.13	34.0	8,281	12.9	78.4	6.9	1.5
5	15-19 months	9.15	10.97	34.80	38.0	11.99	31.5	8,840	26.2	63.0	3.4	6.6
11	20-36 months	7.50	10.70	34.80	46.4	14.30	30.7	8,050	24.0	64.5	5.0	6.0
7	3-4 years	8.70	11.50	40.00	46.0	13.00	28.2	7,063	25.0	60.5	3.8	9.7
6	4-6 years	7.89	11.20	38.70	49.2	14.20	28.8	6,950	21.0	64.2	5.0	8.8
7	6-14 years	7.47	11.10	37.40	50.0	14.86	29.7	6,630	18.5	65.8	3.2	12.0

* Determined from the PCV of the Wintrobe hematocrit. The Microhematocrit PCV leads to higher values due to more complete packing of the erythrocytes.

Table 18. Normal Values in Blood of Several Breeds of Purebred Cattle (Females)

Breed	Age Group	Number of Animals	Ranges and Mean for Each Measurement					Differential Leukocyte Count in Percentage				
			RBC 10 ⁶ /cmm.	Hb. gm. %	PCV	WBC 10 ³ /cmm.	Neut.	Lymph.	Mono.	Eos.	Bas.	
Holstein	mature	13	Min.	7.20	9.25	33.0	4.75	18.0	36.0	3.0	0	
			Max.	10.80	13.25	48.0	12.70	46.0	71.0	8.0	7.0	2.0
			Mean	8.78	10.80	38.0	7.84	32.5	54.3	5.7	5.2	0.6
Jersey	less than one year	23	Min.	7.34	8.45	25.0	5.15	4.0	45.0	0	0	0
			Max.	17.35	13.13	44.0	10.55	43.0	84.0	16.0	3.0	2.0
			Mean	11.20	10.90	32.5	7.76	18.3	72.2	7.6	0.9	0.3
Jersey	mature	58	Min.	4.63	7.75	27.0	3.55	8.0	33.0	1.0	0	0
			Max.	12.50	14.00	50.0	17.45	51.0	85.0	15.0	32.0	2.0
			Mean	8.26	11.21	37.3	7.88	24.7	60.0	4.6	9.7	0.9
Hereford	less than one year	26	Min.	—	9.50	27.0	5.75	12.0	12.0	1.0	0	0
			Max.	—	13.50	50.0	17.80	38.0	80.0	5.0	10.0	2.0
			Mean	—	11.32	37.5	11.14	23.7	68.0	3.2	4.3	0.6
Hereford	mature	50	Min.	—	9.63	31.0	3.20	8.0	33.0	2.0	0	0
			Max.	—	14.25	47.0	12.05	48.0	87.0	8.0	30.0	2.0
			Mean	—	11.83	38.4	7.50	27.9	57.6	4.1	9.4	0.6
Angus	all ages (mostly mature)	23	Min.	6.50	10.38	36.0	5.05	6.0	44.0	2.0	0	0
			Max.	17.90	14.00	49.0	20.10	45.0	85.0	7.0	19.0	2.0
			Mean	9.76	12.00	40.6	9.70	26.9	62.9	4.6	5.0	0.6

Hemoglobin in grams per 100 ml. was determined on the Leitz Photometer using the oxyhemoglobin method.
Differential leukocyte counts were based on 300 cells per count.

lymphocytes decrease again. In Holman's data,⁸⁵ lymphocytes represent only 33 per cent at birth and rose to 72 per cent in 4 months after which there was a slow fall to the adult level of 50–60 per cent at about 2 years of life. In the application of hematology to clinical medicine in the very young bovine, it must be realized that neutrophils will exceed the lymphocytes in number during the first weeks of life. During the period 4–12 months, the neutrophil number is at its lowest and the lymphocytes may contribute 75–80 per cent to the differential count.

ERYTHROCYTIC VALUES IN ADULT CATTLE

Erythrocyte Number, PCV and Hemoglobin. A range of 5 to 10 million erythrocytes per cmm. of blood will encompass nearly all of the normal cattle above 1 year of age. The mean value for red cell count as reported by various investigators (Table 19) is said to be less than 6 million;^{81,84,93,98} between 6 and 7 million;^{12,19,64,67,69,75,76,78,110} between 7 and 8 million;^{97,111,112} and, over 8 million.⁶⁵ The data reported in Tables 17 and 18 show mean counts for mature dairy cattle to be between 7.5 and 8.8 million per cmm. Since then limited data showing lower mean counts have been obtained by a different technician working with cattle from the same dairy herd, *i.e.*, 14 purebred Holstein females, 4–4½ years of age, 6.59 million; and 8 purebred Jersey females, 2–10 years of age, 6.89 million. These latter results are consistent with a large number of reports found in the literature. These observations lend support to the opinion that, insofar as erythrocyte counts are concerned, different results are to be anticipated whenever a change in technical assistance takes place in a clinical laboratory.

The volume per cent of erythrocytes is a much more reliable figure and reproducible results are readily obtainable in the same laboratory by different individuals so long as the speed of the centrifuge and duration of centrifugation are standardized. The mean values for PCV lie between 34 and 38 per cent. It has been stated that with cattle blood³ a centrifugation time of 60 minutes is required to obtain maximum packing of the erythrocytes. This is because of small size of the red cell and absence of rouleaux.

The mean hemoglobin values for cattle, as reported by many investigators, are surprisingly uniform despite the technical difficulties often experienced with clinical hemoglobinometry (Chapter 2). A mean figure of 11.0 grams per 100 ml. of blood is common to several reports.^{70,71,95,107}

Influence of Sex. Scarborough¹⁹ states that bulls show erythrocyte counts 1.0–1.5 million greater than females; Fraser¹¹⁰ reported a mean of 5.69 million for dairy cows and 8.24 million for bulls. McCay⁹⁰ found the mean hemoglobin value of the female of dairy breeds to be $10.9 \pm$

Table 19. Literature References Relative to the Blood Values of Cattle

Reference	No. Cattle	RBC $\times 10^6$	PCV	Hb. gm. %	WBC	Neut. %	Lymph. %	Mono. %	Eos. %	Bas. %
Greatorex ⁸¹	49	5.7 $\pm$ 1.3	37.4 $\pm$ 4.0	12.0 $\pm$ 1.5	9.1 $\pm$ 1.4	12-54 (30)	36-72 (57)	0-8 (2)	2-80 (11)	0 0
Norris ¹¹²	(ave. age 7 yrs.) 27	7.8	—	—	4,900	27.0	54.0	9.0	8.0	0-1.0
Canham ⁷³	35	5.4-8.0	—	—	9,700	33.0	53.0	4.0	9.0	1.0
Fraser ¹¹⁰	—	6.5 $\pm$ 0.74	—	—	7.8 $\pm$ 1.2	30.7 $\pm$ 7.4	50.7 $\pm$ 7.4	7.2 $\pm$ 2.7	11.2 $\pm$ 4	0.24 $\pm$ 0.18
Delaune ⁷⁵	5	6.39	—	—	10,225	25.9	58.1	8.0	7.0	0
Scarborough ¹¹⁹	—	5.0-8.0 (6.62)	—	8.9-11.0	6.0-12.0 (9,250)	20-40 (31.9)	45-65 (55.4)	3-15 (5.2)	3-15 (7.7)	0-1 (0.62)
Reid ⁹⁷	49	7.16	36.48	13.29	—	—	—	—	—	—
Holman ⁸⁴	(Ayrshire) 81	5.95 $\pm$ 0.765	33.7 $\pm$ 4.14	11.3 $\pm$ 1.49	7.03 $\pm$ 1.96	29.1 $\pm$ 9.15	51.4 $\pm$ 11.8	8.32 $\pm$ 2.7	9.87 $\pm$ 11.9	0
Braun ⁸⁷	25	6.66	34.4	11.8	9,263	28.6	52.4	8.9	9.3	0.8
Kohanawa ¹²	—	6.78	—	—	8,210	33.0	51.7	4.3	10.9	0.4
Dimock ⁷⁶	21	4.8-7.9 (6.15)	—	—	2.3-10.6 (5,480)	13.2-45.8 (30.5)	31-76 (54.2)	0.2-3.3 (1.47)	3.9-26.5 (13)	0.1-1.2 (0.6)
Moore ⁹³	15	5.433	—	8.73	6,380	33.2	58.3	2.9	4.33	0.6
Benjamin ⁶⁵	100 (beef)	8.26	—	—	9,094	27.17	61.79	7.11	3.90	—
Ferguson ⁷³	25	4.1-10.0 (6.32)	—	—	3.3-18.7 (8,900)	34.7	41.2	7.9	14.9	0.6
Bel ⁶⁴	35	6.12	—	—	9,570	34.8	42.3	9.5	12.4	1.0
Brody ⁶⁹	7	6.95	36.6	12.7	8,570	32.2	59.2	0.7	6.4	0.5
Josland ¹¹¹	—	6.2-8.9 (7.6)	—	—	4.4-8.0 (5,600)	18-49 (32)	36-66 (53)	0.8 (3)	1-19 (12)	— (0.2)

Other references: Brooks⁷⁰, Byers,⁷¹ Clawson,⁷⁴ Hubbard,⁸³ McCay,⁹⁰ Neal,⁹⁵ Rusoff,^{91,93,100} Allcroft,¹⁰⁷

0.86 and for bulls, 12.8 ± 0.8 grams per 100 ml. of blood. Byers, Jones, and Haag⁷¹ report no significant differences between the sexes in hemoglobin values. Anderson *et al.*⁶³ state that the female cattle, in a single herd constituting their report, had higher hemoglobin levels than the males. Holman⁸⁴ considers differences in blood morphology between the sexes to be unproven.

Breed Differences. McCay⁹⁰ studied 38 Holsteins, 8 Ayrshires, 10 Guernseys and 13 Jerseys and found the mean hemoglobin values to be 10.6, 10.7, 10.4, and 10.5, respectively. He concluded that there was no relationship between the hemoglobin level and the following variables: breed, milk production, fat production, and prolongation of the lactation period. Byers⁷¹ claimed statistically significant differences between the hemoglobin concentration of Holstein and Jersey cattle, the means being 10.6 and 11.3 grams per 100 ml., respectively. On the other hand, Anderson⁶³ found Holsteins to have the highest Hb. value of the various breeds. Greatorcx⁸¹ states that there is a significant difference in hemoglobin and PCV for the breeds and especially for the Jersey breed which he found to have the lowest mean hemoglobin value among Ayrshires, Shorthorn, Guernsey, Friesian, and Jersey breeds. Each breed was from a different farm and thus the influence of management cannot be excluded. Rusoff¹⁰⁰ made monthly observations on the blood values of 5 mature bulls of each of three breeds, all of which were under identical management. The results of this investigation were as follows:

	<i>Jersey</i>	<i>Guernsey</i>	<i>Holstein</i>
PCV	29.5-49.5 (42.3)	34.0-53.5 (46.3)	37.0-50.0 (39.5)
Hb.	9.4-13.3 (11.6)	10.4-14.0 (12.2)	9.9-14.4 (11.6)
RBC	5.1-8.83 (6.55)	5.8-9.12 (7.49)	6.5-9.88 (7.84)
WBC	4.1-15.8 (8.58)	4.2-10.9 (6.44)	4.6-12.6 (7.41)

Influence of Climate and Nutrition. Smith and Kilbourne¹⁰¹ pointed to an observation on erythrocyte counts on cattle in the Washington, D.C. area which led them to the opinion that counts were greater in winter than in summer, the average being 7.0 and 5.0 millions, respectively. Clawson⁷⁴ reporting on blood counts of cattle in Colorado, type not stated but no doubt beef breeds only, observed that in early summer the mean counts were 9.3 million, while in late summer they were 8.6 million. When 11 head were studied at an elevation of 5,000 feet in early summer, their mean count was 8.7 million; following this, they were transferred to a 9,000 foot elevation and in late summer their mean count was 9.2 million, an increase of 500,000 cells per cmm. of blood. Clawson states the effect of altitude would be much greater than appears from the data, since the latter counts were made in late summer when previous experience had shown that red cell number normally falls. Clawson's data indicate an erythrocytic response to reduced

oxygen tension at the higher elevation. Byers⁷¹ observed that pasture feeding did not change the hemoglobin value significantly from barn feeding in a group of 20 cows. McCay⁹⁰ found no differences in total phosphorus, iron and hemoglobin in the blood of 24 cows at the close of the winter months and after 2 months at pasture. Neither was any influence observed of an 18-months' feeding period, with three levels of protein, 16, 20 and 24 per cent, upon the hemoglobin, iron or total phosphorus of the blood of lactating cows. This indicates that the minimum protein intake was adequate for maintenance of hemoglobin levels. Greig and Bayne⁸² investigated the effect of high and low planes of nutrition on the erythrocytic cells of monozygous calves and report a significant difference in favor of the higher nutritional level. Rusoff,¹⁰⁰ in blood studies of mature breeding bulls of the Jersey, Guernsey and Holstein breed, observed an increase in PCV, hemoglobin and total leukocyte count during the summer months when environmental temperature increased over 80° F., although phosphorus values and erythrocyte counts were not affected. Brody,⁶⁹ in studying the influence of ambient temperatures on blood composition, states that moisture loss by sweating in cattle is relatively low and that, when drinking water is cooler than the environment, animals increase their water consumption. Consequently, with rising ambient temperatures, the concentration of blood constituents tends to decrease, provided cool water is available. In their study, hemoglobin level was not influenced significantly by an ambient temperature range of 50°–100° F. Manresa *et al.*,⁸⁹ however, observed a significant influence of environmental temperature on the hemoglobin level of native and imported cattle in the Philippines. They report that the atmospheric temperature bears a close reciprocal but negative relation between hemoglobin indices. The hemoglobin is highest during the coolest months and lowest during warmest months of the year. Observations made every 3 to 4 hours throughout the day revealed hemoglobin levels to follow a regular pattern of being highest in the morning and evenings and lowest in the late morning or early afternoon when atmospheric temperatures were highest. A sample of their data obtained on Indian Nellore oxen follows:

(10 animals 1–3 years of age)						
Time of day	6:30 am	9:00 am	11:30 am	2:00 pm	4:30 pm	
Mean Hb.:	9.41	8.70	8.53	8.70	8.72	
(7 animals 6–10 years of age)						
Time of day:	2:00 am	6:00 am	10:00 am	2:00 pm	6:00 pm	10:00 pm
Mean Hb.:	11.12	10.17	10.2	9.69	10.4	10.45

These workers observed low mean levels of hemoglobin among imported breeds such as the Hereford and Holstein, *i.e.*, 6.76 grams per 100 ml. and 8.28 grams per 100 ml., respectively. European cattle do not thrive

in the tropics and the low hemoglobin levels may be one expression of the effect of such environment on cattle introduced from more temperate zones. The daily fluctuations in the hemoglobin of native cattle probably can be attributed to changes in water balance, more water being consumed as the temperature rises.

Effect of Parturition. Morris⁹⁴ made a detailed study of the blood in a single Shorthorn cow throughout gestation and following calving. Erythrocyte number showed a slight decrease in the latter half of gestation, a marked decrease at 36 hours after parturition and this was followed by a return to normal in 15 days. The hemoglobin levels followed a similar pattern. Van Soest¹⁰³ observed an increase in the hematocrit level at the time of parturition with a gradual decline in the post-partum period. Generally the hematocrit was lower in all cows at 15 to 30 days post-partum than at any time in the study.

Erythrocyte Diameter. Holman^{10a} states that healthy cows, as well as sheep, goats and horses, have a compensatory mechanism whereby animals with a low erythrocyte count have corpuscles above average size and those with high counts have corpuscles below average size. These changes can be demonstrated both by measurements of MCV and mean cell diameter as well as MCH. The occurrence of anisocytosis in normal cattle blood has been mentioned.^{60,80} Fraser¹¹⁰ has given a detailed description of anisocytosis in cattle blood as follows: "The limits of size found in normal cattle are 3.6 μ . to 9.6 μ ., but cells differing from the mean by more than 1.0 μ . are rarely met with, the greater differences occurring usually in young animals. More than half the corpuscles lie between 4.5–5.0 μ . in size. The upper limit might be set at about 7.8 μ . except for a very few cells which occur in animals of all ages and measure between 8.6 and 9.6 μ . These were never found more frequently than one in many fields, and their staining properties resembled those of the other red blood corpuscles. They were not seen in increased numbers in any of the diseased cattle examined, and as nucleated forms were never observed classification is difficult. As they appear to be a physiological occurrence it seems most probable that they are merely macrocytes and have no relation to the large corpuscles of embryonal blood formation; but no evidence was obtained in support of either possibility." Blount⁶⁶ is in agreement with Fraser in that he states that the individual red cell ranges from 4 to 8 μ . but he found the majority to range in size from 5 to 7 μ . Fiennes⁸ states that corpuscular size is influenced by the amount of water in the plasma for when the plasma becomes diluted a portion of the fluid passes into the erythrocyte increasing its size. Others have reported the mean range or mean size to be 5.1 μ .,¹² 5.4–6 μ .,^{18,104} 5.6 μ .,^{10,19} 5.7 μ .,^{84,85} 6.0 μ .,⁷³ and 6.6–7.9 μ .⁸¹

Reticulocytes. Reticulocytes are not found beyond the second day of life in peripheral blood of healthy cattle.

Sedimentation of Erythrocytes. Normal bovine erythrocytes do not participate in rouleau formation, and therefore, little or no sedimentation should be anticipated in the Wintrobe tube during the short period of 1 hour. Ferguson⁷⁷ made observations on the sedimentation rate (Cutler tube) over a 7-hour period on the blood of 22 normal Holsteins varying in age from 3 to 8 years. Calculations from the data presented reveal a distinct trend toward increased rate with decrease in PCV, although the distance of fall was less than 3 mm. in all cases.

<i>No. Cows</i>	<i>Mean PCV</i>	<i>Mean distance of fall in mm. in 7 hours</i>
2	28.8	2.62
6	29.5	2.62
2	30.2	2.46
6	31.5	2.30
2	32.4	2.38
1	33.5	2.17
2	34.3	2.21
1	37.8	1.72

The mean PCV for the 22 cows was 31.32 and the mean sedimentation rate in 7 hours was 2.394. Ferguson concluded that a value over 4 mm. in 7 hours is to be considered as indicative of disease.

Bunce,³ employing standard Wintrobe tubes, recorded the distance of fall of the red cells after 24 hours as 4 mm. maximum and 2.25 mm. minimum for normal cattle. Greator⁸¹ found somewhat higher values and stated the range for both calves and adults to be 1-8 mm. in 24 hours with a mean of 3.0 ± 1.0 with the faster sedimentation being associated with the low normal hemoglobin and PCV values. Rankin⁹⁶ made a study of the sedimentation test in experimental Johne's disease and came to the conclusion that the test is unlikely to have any application in bovine clinical medicine. In reality, the test has not been given a thorough trial with bovine blood, perhaps, mainly because it requires more than a single hour to obtain the results. Therefore, the value of the test in clinical medicine has not been ascertained. Cornelius, Rhode and Bishop¹⁰⁸ have suggested substituting the estimation of seromucoid level in blood plasma for erythrocyte sedimentation rate in sheep and cattle as a measure of the presence and severity of an inflammatory process.

Erythrocyte Fragility. Considerable variation is found in the literature on this subject. Scarborough¹⁹ states that hemolysis begins at a saline concentration of 0.60 and is complete at 0.40; Rossouw¹²⁷ gives 0.65 per cent as the starting point of hemolysis. Holman⁸⁵ found resistance of the erythrocyte to be greatest at birth, 0.346 per cent saline; followed by a decrease in resistance reaching a point of greatest fragility in calves at 5 months of age, 0.532 per cent saline; from this point resistance increased

up to 2 years of life, 0.483 per cent saline; and, for mature cows an average of 0.474 per cent saline for complete hemolysis is given. Greator^{80,81} also observed variations in erythrocyte fragility with age, namely: 0.3–0.35 per cent saline during the first 4 weeks of life; decreasing to 0.4–0.45 during the next 4 months; with hemolysis occurring at a saline concentration of 0.50 per cent from the twentieth week onward during the first year of life and a mean of 0.45 per cent for adult cattle.

LEUKOCYTIC VALUES IN ADULT CATTLE

Leukocyte Counts, Total and Differential. The mean total leukocyte count of the adult cow is between 7,000 and 9,500 per cmm. of blood. However, in aged cows the mean total count may be lower.⁹² The affect of age on the percentage distribution of neutrophils, lymphocytes and eosinophils, especially during the first year of life, has been touched upon. Moberg⁹² has reported on the normal white blood picture of 203 sexually mature female cattle; Table 20 is taken from Moberg's work. These data demonstrate that with advancing age a decline in total count as well as in absolute numbers of neutrophils and lymphocytes occurs and at the same time the relative and absolute numbers of the eosinophil increase. Moberg was of the opinion that reproductive phenomena, like pregnancy and lactation, may be responsible for these changes.

The decrease in neutrophils and increase in eosinophils with age observed by Moberg⁹² and seen also in Table 17 may reflect a sluggish pituitary-adrenal action in the bovine. It is well known in clinical medicine that the bovine does not respond to infections with leukocytosis of the magnitude commonly found in the canine and feline. A shift in neutrophils to 50 per cent or above with occurrence of a few bands or younger forms is considered indicative of a stress reaction to bacterial infection with or without a significant increase in total count; or a distinct left shift without significant neutrophilia or leukocytosis.

Occurrence of Young Neutrophils. Band neutrophils are almost nonexistent in peripheral blood of the bovine in health. Among the 193 cattle of all ages and including both beef and dairy types (Table 18) band forms were found in only 2 calves and only to the extent of 2 and 4 per cent. Greator⁸¹ stated that in calves up to 1 year of age young forms of the neutrophil are rare and band forms do not exceed 4–5 per cent; in adults, bands 0.4–2.0 per cent. Blount,⁶⁶ on the other hand, stated that calves may show 8–9 per cent metamelocytes and that 1 to 2 per cent may occur in adults. Obviously, this investigator did not employ a rigid distinction between the mature neutrophil and the true young forms. Fraser¹¹⁰ states that the Schilling Hemogram should be

Table 20. Total and Differential Leukocyte Counts, Absolute Number and Percentage, and Lymphocyte/Neutrophil (L/N) Ratio in Sexually Mature Cattle (from Moberg)¹³

Group	No. of Cows	Total Count	Neutrophils	Lymphocytes	Monocytes	Eosinophils	L/N Ratio
Heifers, virgin.	48	11,116 ± 406 (100%)	2,466 ± 142 (22.1%)	7,768 ± 300 (69.9%)	188 ± 17 (1.7%)	674 ± 27 (6.1%)	3.2
Heifers, pregnant.	51	9,286 ± 330 (100%)	1,995 ± 122 (21.5%)	6,192 ± 264 (66.7%)	175 ± 15 (1.9%)	904 ± 20 (9.7%)	3.1
Cows, having calved once.	33	8,225 ± 331 (100%)	2,131 ± 174 (25.9%)	5,062 ± 230 (61.5%)	121 ± 12 (1.5%)	878 ± 24 (10.7%)	2.4
Cows, having calved 2-3 times.	35	7,795 ± 407 (100%)	1,901 ± 110 (24.4%)	4,803 ± 295 (61.6%)	115 ± 14 (1.5%)	906 ± 31 (11.6%)	2.5
Cows, having calved 4 or more times.	36	7,106 ± 322 (100%)	1,838 ± 98 (25.9%)	4,250 ± 321 (59.8%)	120 ± 10 (1.7%)	952 ± 22 (13.4%)	2.3

modified in its application to the neutrophil cells of cattle. The distinction between band forms and older cells is usually obvious from the nuclear lobulation, but the older cells are not necessarily segmented; at times there is some difficulty in deciding whether a cell shall be placed in the band form or mature cell classification (see Chapter 3, p. 99).

Effect of Estrus. Moberg⁹² observed limited changes in the white blood cell picture in connection with estrus. Cows showed a slight increase in total white count which was due to an increase in neutrophils, while the eosinophils were decreased on the day of heat and the first day thereafter. In heifers, Moberg reported a tendency toward lymphopenia and eosinophilia as well as neutrophilia during estrus.

Effect of Pregnancy. The total leukocyte count increases up to the fourth month and then slowly recedes.⁹² The increase in total count is not great being limited to less than 1,000 cells per cmm. of blood and, therefore, in routine blood studies such slight changes would go unnoticed. The neutrophils increased during the first half of pregnancy, fell and increased again until parturition. The lymphocytes, after increasing up to the third month, showed a continuous decrease throughout the remainder of gestation. This drop in lymphocytes seems to be the primary reason for the fall in the total white count in advanced gestation. The eosinophils exhibited no definite trend for several peaks and low points interchange throughout gestation.

Effect of Parturition. The blood picture is typical of a mild or moderate response to stress.^{101a} According to Moberg,⁹² a leukocytosis occurs due to an increase of neutrophils of about 100 per cent, the eosinophils fall by 50 per cent and the number of circulating lymphocytes, which have reached their lowest level during gestation, do not undergo any considerable change in number during parturition. Others^{87,88,91} have indicated a distinct drop in lymphocytes on the day of parturition, with the L/N ratio below unity for at least 36 hours after calving.⁸⁷ A gradual return of the leukocytic picture to normal occurs over a period of 5 days. Injection of ACTH or epinephrine into adult female cattle induces changes in the leukocytic picture similar to those occurring during parturition but of a more marked nature^{86,91} (also see Chapter 10, p. 332).

Effect of Drying-off of Lactation. Morris,⁹⁴ in studying the hematology of a single Shorthorn cow during normal pregnancy and parturition, states that a slight leukocytosis occurred when the cow was dried-off and a second leukocytosis occurred 2 weeks prior to parturition. The leukocytosis in both cases was mainly due to an increase in neutrophils, but during drying-off there was a definite increase in eosinophils, while prior to parturition there was a decrease in eosinophils. The rise in eosinophils during the drying-off period may be a manifestation of a mild allergic response to the retention of milk protein in the udder.

That a pronounced allergic reaction is possible in some cows when drying-off has been reported.⁶⁸

The Myeloid-Erythroid Ratio. Winqvist¹⁰⁴ conducted an extensive study of bone marrow in bovine embryos, calves and adult cattle. He found cellular composition of marrow from various bones to be quite similar, but concluded that at least 3,000 cells should be differentiated for

Table 21. The Cellular Composition* of the Bone Marrow of Adult Cattle as Reported by Different Investigators. Adapted from Winqvist¹⁰⁴

	Hölzel	Marcate	Hjärre	Calhoun ¹	Winqvist ²
Myeloblast . . .	0-3	—	1.5-4.0	—	—
Progranulocyte . .	1-4	2.95	0.5-3.0	1.51 (0.0-6.8)	0.79 (0.2-1.4)
Neut. myelocyte . .	5-9	5.85	3.0-9.6	19.39 (10.4-32.0)	2.90 (1.0-5.0)
Eos. myelocyte . .	0.5-5	5.0	—	6.69 (1.8-10.4)	0.73 (0.1-1.4)
Neut. metamyelocyte	1-4	11.8	3.0-13.5	include myelo.	4.14 (1.3-7.4)
Eos. metamyelocyte	1-4	5.79	—	—	2.09 (0.7-3.4)
Neut. band . . .	10-21.5	—	7.5-18.5	—	8.2 (5.0-12.8)
Neutrophil . . .	10-21	—	6.5-19.5	5.73 (1.2-12.2)	11.46 (4.9-19.0)
Eosinophil . . .	5.5-18	—	—	1.92 (0.0-7.6)	5.67 (2.4-10.8)
Basophil . . .	—	—	—	0.34 (0.0-1.0)	0.19 (0.0-0.8)
Lymphocyte . . .	3.5-11	3.69	5.0-20.0	6.68 (1.4-16.8)	10.69 (5.6-16.3)
Monocyte . . .	0-1	8.86	0-1.0	2.64 (0.0-7.6)	0.16 (0.0-0.4)
Plasma cell . . .	0-2	0.59	—	0.79 (0.2-2.0)	0.99 (0.4-2.3)
Reticulum cell . .	0-0.5	—	—	—	1.27 (0.2-2.9)
Megakarocyte . .	0-1.5	0.52	—	—	0.05 (0.0-0.2)
Stem cell . . .	—	4.23	—	2.14 (0.0-5.0)	0.43 (0.2-0.8)
Prorubricyte . . .	1-4	2.36	0-1.0	—	1.23 (0.6-1.7)
Rubricyte . . .	5.5-10	29.13	30-62.5	30.26 (11.8-42.8)	25.73 (16.3-30.8)
Metarubricyte . .	19.0-30	19.25	—	21.69 (7.2-39.2)	23.28 (10.4-35.3)
M:E ratio . . .	—	0.62	0.7	0.676 (0.27-2.5)	0.79 (0.4-1.84)

¹ = 12 mature cows, rib marrow

² = 5 mature cows, sternal marrow

*Cell distribution stated in percentage.

critical determination of differential distribution of various cell types. However, due to the time consuming nature of differentiation of such large numbers of cells, Winqvist generally was satisfied with counts based on 1-2 thousands cells. He found the M:E ratio to be 0.47 ± 0.06 in calves 3 to 36 days old; 0.64 ± 0.13 in cattle from 2 months to 10 years of age; and, in 5 adult cows the mean ratio was 0.79 ± 0.18 (Table 21). Calhoun¹⁰⁵ found a similar mean M:E ratio in rib marrow of 12 cows, i.e., 0.676:1.0.

Thrombocytes. The thrombocyte number per cmm. of blood in calves has been found to vary from 152,000 to 1,229,000^{81,104,110} and in adults from 74,000 to 740,000.^{81,110} They generally appear in clusters consisting of a few to many with complete loss of cytoplasm but may occur as

singly dispersed, circular bodies reaching the size of erythrocytes.^{66,110} These giant forms have a delicate, but distinct cell membrane enclosing a pale-staining cytoplasm and deeply staining granules.

Icterus Index. Cattle on green feed develop a yellow color in their blood plasma due to the plant pigments which are collectively referred to as carotinoids. The pigment in bovine plasma has been shown to be mainly carotin.^{15a} The color of the plasma due to pigmentation of plant origin may measure as much as 25 units on the icterus index scale. Cattle on dry feed have almost a colorless plasma for the bilirubin level is normally low. The bovine liver possesses a very large fundamental reserve for excretion of bilirubin for even in generalized liver disease the maximum serum bilirubin concentration did not exceed 3.5 mg. per cent.⁷⁹

Specific Gravity. The specific gravity shows a positive correlation with hemoglobin concentration⁸⁵ and, from birth to 30 months of age, specific gravity was found to range from 1.046 to 1.0550; the mean value for adult cattle is given as 1.0520. Greator⁸¹ reports 1.0520 for calves and 1.0490 for adult cattle.

THE SHEEP

Erythrocytic Series

<i>Erythrocytic Series</i>		<i>Extreme Range</i>	<i>Ave. Range</i>
Erythrocytes		8.0-16.0	10.0-14.0
Hemoglobin		8.0-16.0	11.0-13.0
PCV		24.0-50.0	32.0-40.0
MCV		23.0-48.0	30.0-38.0
MCH		9.0-12.0	9.5-11.0
MCHC		29.0-35.0	31.0-33.0
Reticulocytes		0	0
Sedimentation rate/1 hour		0.0-3.0	2.0
RBC diameter		3.2-6.6	4.0-5.0
Resistance to hypotonic (min.)		0.58-0.76	0.45-0.65
saline (max.)		0.40-0.55	
M:E ratio =		0.77-1.68:1.0.	

Leukocytic Series

	<i>Range</i>	<i>Ave.</i>
Leukocytes	4-12,000	7-10,000
Band neut.	0-2	0.5
Neutrophil	10-50	30.0
Lymphocyte	40-75	62.0
Monocyte	1-6	2.5
Eosinophil	1-10	4.5
Basophil	0-3	0.5

Other Data

Thrombocytes	2.5-7.5 × 10 ⁵	4.0
Icterus index	Normally less than 5 units	
Specific gravity	1.038-1.065	1.052

mean = 1.103:1.0 from Grunsell¹¹⁷

The hematology of the ovine is similar to that of the bovine with the exception of size and number of erythrocytes. Due to the type of husbandry, sheep harbor gastrointestinal parasites much more commonly than cattle. Literature references on normal blood values, summarized in Tables 22 and 23, no doubt reflect to some extent the influence of parasitism. Several authors state that the sheep were in good physical condition but not necessarily free from internal parasites. Holman¹²³ observed variations in mean hemoglobin values in adult sheep as follows: spring, 9.6 grams; summer, 11.7 grams; autumn, 10.6 grams; and, winter,

11.7 grams. He attributed the low levels of spring and fall to inanition. Hawkins¹²² observed over a 5-year-period a decided decrease in nematode egg counts in fecal samples from sheep and an increase in the concentration of hemoglobin during the winter months in ewes and yearling lambs. In late February and March, there were increased egg counts and a lowering of hemoglobin. Grunsell¹²⁰ concluded that a diet providing half maintenance had no effect on hematopoiesis in worm-free sheep; however, when parasites were present, a maximum effect in lowering hemoglobin and PCV values was observed in sheep on a low plane of nutrition. A significant association between lower erythrocyte counts and a rise in worm burden was found in late winter and early spring. However, in the summer months, the period of nutritional abundance, the red cell counts increased thus suggesting that a high level of nutrition partly offsets the effect of the worm burden. As early as 1907, Giltner¹¹⁶ reported low hemoglobin levels and red cell counts in parasite-infested sheep and asserted that effects of parasitism were partially overcome by providing a more generous and easily digested ration.

Effect of Age on Blood Values. Data from the literature (Tables 22 and 23) have been arranged as much as possible on an age basis. Several investigators^{110,111,112,114,126} have compared the hematology of lambs and adult sheep. The mean erythrocyte counts in lambs up to 1 year of age generally have been reported as being between 11 and 13 million per cmm. of blood and 1-2 million less in adults. Nothing was found relative to the changes occurring in erythrocyte size and number during the early period of life, although Holman¹²³ reported the mean MCV of 15 lambs under 1 month of age to be 35.5 as compared to 27.4 for 99 adult sheep. The observation was claimed to be statistically significant. The mean MCV value of 27.4 for adult sheep is considerably less than mean values reported by other investigators.

Grunsell¹²⁰ correlated blood values with age (Table 24) but placed all lambs in a single group up to 1 year of age. He pointed out that sheep under 2 years of age carried a higher worm burden and that such sheep are acknowledged to be more susceptible to the effects of helminth infestation. These facts may account for the lower RBC, PCV and hemoglobin levels in his data during the first 2 years of life. Total leukocyte count did not appear to be influenced by age, although in the data presented by Grunsell, a gradual rise in neutrophils and eosinophils with age can be detected. Holman¹²³ found eosinophil counts to average 1.1 per cent in lambs as compared to 4.2 per cent in adult sheep, and Fraser¹¹⁰ reported similarly.

The neutrophil count is highest at birth, exceeding 50 per cent of the differential, and within a few weeks drops to approximately mature levels.^{110,126} The band neutrophil contributed 1.5 per cent to the differ-

Table 22. Literature References on RBC and WBC Counts in Lambs and Adult Sheep

Reference	No. Animals	Breed	Age	RBC $\times 10^6$	WBC $\times 10^6$	Neut. %	Lymph. %	Mono. %	Eos. %	Bas. %
Fraser ¹¹⁰	—	—	1-7 days	9.0-14.0 (11.3)	4-13 (7.9)	33-74 (54)	20-63 (41)	1-8 (3.6)	0-2.4 (0.6)	0-1 (0.1)
Josland ¹¹¹	—	—	4-8 mos.	11.8 $\pm$ 1.2	10.7 $\pm$ 1.2	22 $\pm$ 5	72 $\pm$ 6	3 $\pm$ 1	3 $\pm$ 1	—
Norris ¹¹²	28	—	Lambs	10.3-20.0 (13)	2.9-9.3 (5.5)	13-59 (34)	30-72 (55)	0-39 (9)	0-7 (1)	0-1 (0)
		Egyptian	1-60 days	9.08-12.78 (10.92)	10.0	64.5	44.0	1.2	0.2	0.1
		Egyptian	2-12 mos.	9.24-11.92 (10.59)	8.5	39.0	55.0	3.0	2.5	0.5
Reda ¹²⁶	60	Egyptian	1-2 yrs.	9.02-10.45 (9.73)	9.2	43.0	48.5	4.3	4.0	0.2
		Egyptian	2-5 yrs.	7.6-9.21 (8.58)	8.0	41.0	50.0	3.0	5.7	0.3
Hackett ¹²¹	200+	Suffolk	Lambs and ewes	10.89	9.95	29.9	64.6	2.5	2.4	0.5

Fraser ¹¹⁰	—	—	Adult	7-12 (10.3)	5-12 (8)	27-46 (39)	40-63 (52)	2-5 (3.2)	0-22 (4.9)	0-2.8 (0.7)
Scarborough ¹¹⁹	—	—	Adult	8-12 (10.38)	5-10 (7.84)	20-45 (35.7)	50-70 (56.9)	1-8 (6)	1-7 (2.5)	0-2 (0.4)
Kohanawa ¹²	—	—	Adult	10.275	10.45	31.8	57.7	1.8	8.7	0.02
Holman ¹²³	100-116	Scottish Hill	Mostly Adult	6.2-15.5 (11.5)	1.1-17.5 (9.2)	11-47 (24)	41-83 (67.3)	0-13 (2.3)	0-15 (4.2)	0-3 (0.5)
Todd ¹²⁹	55	Southdown	Preg. ewes	8.27-15.5 (12.65)	3.15-11.88 (7.26)	10-55.9 (27.6)	34.8-77.0 (62.4)	0.5-7.5 (2.4)	1.8-25.0 (6.2)	0-4.0 (1.4)
	35	Hampshire	Preg. ewes	9.52-17.8 (12.47)	4-13.62 (7.74)	15-49.3 (28.5)	45.8-77.5 (62)	0.3-6.5 (2.2)	1.0-18.5 (5.5)	0.5-4.5 (1.9)
Josland ¹¹¹	—	—	Adult	10.5±0.9	7.0±1.15	29±6	62±6	3±2	6±3	0.2
Norris ¹¹²	22	—	Adult	9.6-16.4 (11.6)	3.0-8.2 (5.3)	27-71 (52)	23-51 (39)	1-21 (6)	0-17 (1)	0-1 0
Hudson ¹²⁵	20	Hampshire	11-36 mos.	4.8-12.2 (8.9)	3.2-10.2 (5.1)	37.0 (1.0% band)	52.0	2.5	7.2	0.5

Table 23. Literature References on Erythrocytic Series in Sheep

Reference	No. Animals	Breed	Age	$RBC \times 10^6$	PCV	Hb. gm. %	MCV	MCH	MCHC
Andrew ¹¹³	9	—	2-8 mos.	11.0-14.8 (12.4)	26-36 (33)	10-12 (11.3)	21.6-31.2 (26.5)	8.1-10.0 (9.1)	30.5-40.0 (34.2)
Becker ¹¹⁴	6	Corriedale	1-14 wks.	10.9-13.7 (12.23)	37.9	12.09	27-36 (31)	8.6-11.0 (9.8)	29.2-34.1 (31.5)
	6	Hampshire Dorset	Yearlings	9.6-13.3 (11.88)	97.0	12.23	27-36 (31)	9-11.3 (10.1)	30.6-34.2 (32.2)
Hackett ¹²¹	200+	Suffolk	Lambs and ewes	10.89	35.0	10.78	32.1*	9.9*	30.8*
Holman ¹²³	100-114	—	Mostly adults	6.2-15.5 (11.5)	22-39 (30.5)	8.6-15.8 (12.4)	19-35 (27.4)	10.8*	41.2
Todd ¹²⁹	55	Southdown	Preg. ewes	8.27-15.51 (12.65)	32.3-47.2 (40.38)	10.3-16.0 (13.4)	23.6-48.6 (32.23)	10.6*	33.2*
	35	Hampshire	Preg. ewes	9.52-17.8 (12.47)	31.3-45.2 (38.35)	10.4-14.7 (12.34)	26.7-41.9 (39.07)	9.9*	32.2*
Becker ¹¹⁴	6	Corriedale Hampshire Dorset	Mature	10.1-13.2 (11.63)	38.8	12.91	28-37 (33.8)	8.8-11.9 (10.8)	30.8-33.3 (32.1)

* Estimated from author's data.

ential leukocyte count in sheep under 3 months of age, about 1.0 per cent in blood taken between 3 and 8 months, and about 0.5 per cent in the blood of sheep over 9 months of age.¹²³ Hackett¹²¹ found a significant downward trend with time in the total number of leukocytes which was attributed to a decrease in lymphocyte values; no change with time was observed in the monocyte, eosinophil, and basophil numbers.

Table 24. Erythrocyte and Leukocyte Values in Sheep of Different Ages (Grunsell¹²⁰)

Item	No. of Sheep	Age in Years						
		Under 1	1-2	2-3	3-4	4-5	5-6	6-7
PCV	194	31.07	31.23	34.10	33.55	34.33	35.12	33.91
Hemoglobin (Sahli) . .	192	9.65	9.62	10.33	10.17	10.23	9.41	10.22
RBC	44	10.72	10.54	11.58	10.82	11.55	10.93	10.74
MCHC	189	31.18	31.03	29.95	30.29	30.00	28.12	30.48
MCV	44	36.20	37.70	35.50	37.80	38.50	38.40	38.69
MCH*	44	9.0	9.1	8.9	9.4	8.9	8.6	9.5
WBC	44	7,872	6,419	7,772	6,536	7,255	7,310	6,362
Band neut.	44	35 (0.28)	50 (0.78)	29 (0.37)	46 (0.70)	17 (0.23)	31 (0.42)	41 (0.64)
Neutrophil	44	2,274 (28.9)	2,317 (36.1)	2,367 (30.4)	2,437 (37.3)	1,880 (25.9)	2,574 (35.2)	2,591 (40.7)
Lymphocyte	44	4,881 (62.0)	3,684 (57.4)	4,781 (61.5)	3,761 (57.5)	4,894 (67.4)	4,154 (56.8)	3,238 (50.9)
Monocyte	44	586 (7.4)	233 (3.6)	418 (5.4)	164 (2.5)	222 (3.0)	300 (4.1)	281 (4.4)
Eosinophil	44	96 (1.2)	135 (2.1)	177 (2.3)	128 (1.9)	242 (3.3)	251 (3.4)	211 (3.3)

* MCH estimated from author's data.

A limited number of blood determinations were made on a total of 12 growing lambs between 60 and 240 days of life. These lambs were raised on boards, they were fed chopped alfalfa hay and supplements, and maintained parasite-free.

From the results of this study, summarized in Table 25, it would appear from the decreasing MCH and MCHC values that these lambs were not receiving adequate iron; also, the erythrocyte counts were 1-2 million cells below the means commonly reported for lambs.^{110,112,113,114} Britton¹¹⁵ published ranges and means for hemoglobin and PCV found among 134 lambs maintained on several different feed conditions in the Davis, California area. He stated that the different rations did not influence the Hb. and PCV levels significantly. The mean PCV for the 134 lambs was 29.1, which is considerably lower than the mean for parasite-free lambs. The mean hemoglobin was reported to be 12.2

grams but this would provide a mean MCHC of 41.9 per cent and, therefore, suggests erroneously high Hb. values. The only other investigator reporting such a high MCHC value (41.2 in adult sheep) is Holman.¹²³ Both reports as far as MCHC is concerned would appear to be in error.

Erythrocyte, PCV and Hemoglobin Values in Adult Sheep. The mean erythrocyte count in adult sheep is between 11.0 and 12.0 million but individual sheep may have counts as low as 8.0 million and as high as 15.0 million or greater.^{112,123,129} Mean PCV values as reported for sheep in Great Britain fall between 30 and 35,^{120,123} while for sheep in the U.S.A. mean values fall between 35 and 40.^{114,121,128,129} The mean hemo-

Table 25. Blood Morphology of Growing, Parasite-free Lambs

Number of determinations	Age in Days	MEAN VALUES												
		RBC	PCV	Hb.	MCT	MCH	MCHC	WBC	Differential Leukocyte Count in Percentage					
									Neut.	Lymph.	Mono.	Eos.	Bas.	
3	60-80	10.76	35.7	12.9	33.2	12.0	36.1	5.85	36.3	54.0	8.0	1.7	0	
8	80-100	10.53	31.9	10.9	30.3	10.3	34.2	5.99	27.3	61.5	8.0	2.8	0.4	
8	100-120	10.68	33.3	12.1	31.2	11.3	36.3	6.94	30.9	59.4	8.3	1.4	0	
4	120-140	9.75	34.9	12.2	35.8	12.5	35.0	10.20	41.3	53.0	5.3	0.4	0	
6	140-160	12.01	36.5	12.1	30.4	10.0	33.2	7.86	24.0	69.5	4.7	1.8	0	
6	160-180	10.52	34.3	11.1	32.6	10.5	32.4	7.80	18.7	71.5	6.0	2.8	1.0	
8	180-200	10.74	35.7	11.3	33.2	10.5	31.6	9.01	24.4	64.4	6.8	3.4	1.0	
1	200-220	10.55	38.0	10.8	36.0	10.2	28.4	6.50	15.0	75.0	7.0	2.0	1.0	
3	220-240	10.12	34.1	9.5	33.7	9.4	27.8	9.13	23.0	71.3	3.7	1.7	0.3	
47	60-240	10.63	34.9	11.4	32.9	10.7	32.7	7.69	26.8	64.4	6.4	6.0	0.4	

globin value is between 11.0 and 12.0 grams per 100 ml. of blood but in individual sheep, levels may reach 16.0 grams.^{123,129} The effect of high altitude in raising the hemoglobin concentration has been reported by Watson.¹³⁰ Sheep at sea level in Peru were observed to have hemoglobin concentrations of 8.0 to 10.0 grams; after 3 to 4 months at altitudes of 11 to 15 thousand feet, the hemoglobin ranged from 12 to 16 grams. Hemoglobin has been observed to decrease appreciably during and subsequent to lambing.¹²¹ Allcroft¹⁰⁷ states that hemoglobin values of sheep and cattle are similar in England and Wales; the mean is 11.5 grams with an extreme range of 8-17 grams and a common normal range of 9.5-13.5 grams.

Influence of Sex and Breed.—No evidence has been found in the literature to the effect that significant sex or breed differences exist.^{114,123,126}

Erythrocyte Diameter. Individual erythrocytes may be as small as 3.2 to 3.8 microns in diameter^{109,110,123,125} or as large as 6.5 microns or

greater.^{109,110,125} Holman¹²³ made Price-Jones curve studies on three sheep and observed individual cells to vary from 3.5, to 6.0 microns in diameter with the mean sizes for the 3 sheep being 4.5, 4.7, and 4.9 microns. Richert¹⁸ gives a range of 4.8 to 5.2. Others have reported the mean size to be 4.1 μ ,¹² 4.2 μ ,¹²⁵ 4.9 μ ,¹⁰⁹ and 5.0 μ .^{10,19,110}

Reticulocytes. A few reticulocytes may be found at birth but they generally disappear within 48 hours and are not found thereafter in peripheral blood in health.¹¹⁰

Sedimentation of Erythrocytes. Erythrocyte rouleaux may be found to a limited extent and, therefore, some sedimentation may be anticipated. When the erythrocyte sedimentation level is observed at the end of 1 hour, 1 to 2.5 mm. of fall may be noted¹²⁶ and, when observed after a period of 24 hours, from 3 to 10 mm. of settling may have taken place.^{3,123} Estimation of plasma seromucoid level may be a more useful means in sheep and cattle for evaluating the severity of an inflammatory process.¹⁰⁸

Erythrocyte Fragility. It is generally stated that hemolysis begins at 0.65 per cent saline^{19,123,125} but Rossouw¹²⁷ found hemolysis to begin at 0.76 per cent saline. Wirth²³ has given 0.80 as a possibility for beginning hemolysis. Complete hemolysis is reported to occur from 0.55¹²⁵ down to 0.46¹⁵ or lower.^{11,19,123}

Leukocyte Counts, Total and Differential. The normal range for total count is similar to that of the cow, namely, 4 to 12,000; considerable disagreement exists regarding the mean count but most investigators have reported it to be between 7 and 8,000. Holman¹²⁴ regards a count of 13,000 as slight leukocytosis and 20,000 as marked leukocytosis. He considers 47 per cent neutrophils as a relative neutrophilia and an absolute count of 5,600 neutrophils or greater as an absolute neutrophilia; a relative neutropenia when the differential count shows 11 per cent or less of neutrophils, and absolute neutropenia when the actual count of neutrophils falls below 700 per cmm. of blood. He classifies as a slight neutrophilic reaction an increase of 2 per cent bands or 16 per cent neutrophils between counts on 2 successive days. The occurrence of 6 per cent band forms he regards as a slight shift to the left, while a marked shift is indicated when metamyelocytes constitute one-third of the neutrophil cells.

The Myeloid-Erythroid Ratio. Grunsell¹¹⁷ has studied aspirated marrow taken from the sternum of 10 Cheviot ewes between ages of 3 to 6 years (Table 26). He has published a detailed description of marrow cells of sheep¹¹⁸ and has investigated the response of the marrow to anemia.¹¹⁹

Thrombocytes. Fraser¹¹⁰ states that in lambs the range was found to be 540,000 to 700,000, with a mean of 620,000; adults, 250,000 to

750,000, with a mean of 490,000. Holman¹²³ reports a range of 75,000 to 575,000 with a mean of 290,000. Tocantins²¹ gives a range of 284,000 to 659,000, with a mean of 441,000.

Icterus Index. The plasma color is not affected by the yellow pigments of the ration as is the case with cattle.^{15a} Thus, icterus index normally does not exceed 5 units; in dehydration it may reach 7.5 units.

Specific Gravity. Very limited information places the range between 1.038 and 1.065 with means of 1.050 or 1.052.^{18,19,123,125}

Table 26. Nucleated Cells in Aspirated Marrow of the Sheep¹¹⁷

<i>Cell Type</i>	<i>Range (%)</i>	<i>Mean (%)</i>	<i>Mean Cell Size in Microns</i>
Haemocyto blasts	0.0- 0.2	0.005	33.00
Myeloblast	0.1- 1.4	0.47	19.18
Progranulocyte	0.0- 0.6	0.24	19.60
Neut. myelocyte	1.5- 8.1	4.60	20.30
Neut. metamyelocyte	2.1- 6.0	4.33	14.63
Band	10.6-22.8	16.38	—
Neutrophil	1.4- 6.9	4.58	—
Eos. myelocyte	3.3- 6.2	4.85	20.57
Eos. metamyelocyte	7.8-15.0	10.84	15.87
Eosinophil	0.8- 5.1	2.80	—
Basophil	0.3- 2.3	1.04	15.73
Total myeloid		50.13	
Rubriblast	0.5- 1.4	0.96	15.60
Prorubricyte	1.5- 4.9	3.30	13.40
Rubricyte	27.2-37.3	31.86	9.19
Metarubricyte	4.9-15.1	9.91	5.37
Total erythroid		46.03	M:E = mean 1.103-1.0*
			range 0.77-1.68:1.0
Plasma cell	0.0- 1.0	0.35	* Grunsell has included the
Lymphocyte	0.6- 3.1	1.73	plasma cells etc. in the
Monocyte	0.0- 0.7	0.26	myeloid series to obtain
Mitotic forms	0.2- 1.6	0.84	the above ratios. Actual
			M:E mean value is 1.089:
			1.0.

THE GOAT

Normal values in the goat are based on extremely limited data. The literature is presented in Table 27.

Erythrocytes, PCV, and Hemoglobin. The hematology of the goat differs from that of the cow and sheep mainly in the size and number of erythrocytes. From the limited data available, it appears that the ranges and means for hemoglobin and PCV are similar to the levels in the cow and sheep. Mean RBC number is in the range of 14-16 million cells per cmm. of blood for the adult goat and may reach 20 million or greater in the young. Mean hemoglobin range is 11-12 grams and for PCV, 32-38 per cent.

Erythrocyte Diameter. The smallest mean size reported is 3.1

Table 27. Literature References on the Blood of Goats

Reference	No. of Goats	Age and Sex	RBC 10^6	PCV	Hb. gm. %	MCV	MCH	MCHC	WBC 10^3	Differential Leukocyte Count in %			
										Neut.	Lymph.	Mono.	Eos.
Mohler ³²	—	—	9,976	—	—	—	—	—	9,200	—	—	—	Bas.
Warthin ¹³³	—	—	16.00	—	—	—	—	—	8,000	—	—	—	—
Reichert ¹⁸	—	—	—	34.72	11.96	—	—	32.4*	—	—	—	—	—
Wells ²²	2	Adults	14,974	—	—	—	—	—	7,300	—	—	—	—
	2	Kids	20,860	—	—	—	—	—	12,525	—	—	—	—
Fish ¹³¹	1	M 15 mos.	15,225	—	—	—	—	—	6,158	43.5	45.9	6.08	3.7
	2	Castrate 15 mos.	13,911	—	—	—	—	—	12,141	41.3	44.1	3.38	9.8
Burnett ⁴	—	—	9.0-19.0	—	—	—	—	—	8-12,000	—	—	—	—
Emmons ⁷	—	—	15.00	38.0	—	24.6	—	—	—	—	—	—	—
Kohanawa ¹²	—	—	17,329	—	—	—	—	—	12,210	34.3	57.4	2.2	6.1
Scarborough ¹⁹	—	—	9.0-19.0 (14,420)	—	—	—	—	—	6-15,000 (12,060)	35-56 (40)	34-74 (48)	1-4 (2)	0.5-7.4 (2.8)
	2	—	17.33	33.2	11.4	19.0	7.0	35.0	7,400	36.5	58.0	2.0	3.5
Wintrobe ^{22a}	—	—	13.0-17.0	—	10.9	—	—	—	8-16,000 (12,000)	30-45 (36.5)	49-72 (55)	4.5 (4.5)	3-8 (5)
Wirth ²³	—	—	—	—	—	—	—	—	—	—	—	—	—

* Estimated from author's data.

microns.¹² Burnett⁴ cites 5 authors who reported means varying from 3.5 to 4.25 microns. Reichert¹⁸ gave a range of 3.2 to 4.2, and others gave the mean as 4.07 and 4.1.¹⁰ Single large erythrocytes having a diameter up to 6–7 microns may occur.¹⁰⁴

Reticulocytes. None are found in peripheral blood in health.

<i>Erythrocytic Series</i>	<i>Range</i>	<i>Ave.</i>	<i>Leukocytic Series</i>	<i>Range</i>	<i>Ave.</i>
Erythrocytes	12.0–20.0	15	Leukocytes	6–16,000	12,000
Hemoglobin	8.0–14.0	11	Band neut.	0–2	0.5
PCV	24–48	35	Neutrophil	30–48	36.5
MCV	18–24	20	Lymphocyte	50–70	55.0
MCH	4.5–7.0	—	Monocyte	1–4	2.5
MCHC	30–35	32	Eosinophil	3–8	5.0
Reticulocytes	0	0	Basophil	0–2	0.5
<i>Other Data</i>					
Sedimentation rate.			Thrombocytes =	2.5–5.0 × 10 ⁵	
1 hour	0	0	Icterus Index =	less than 5 units	
RBC	4.0	—	Specific gravity =	1.042–1.062	
Resistance to hypo-					
tonic saline (Min.)	0.74	—			
(Max.)	0.60	—			

$$\text{M:E ratio} = 0.69:1.0^{104}$$

Sedimentation of Erythrocytes. Rouleau formation is absent in health⁷ and, therefore, no sedimentation takes place within the first hour. However, 2.0–3.0 mm. of settling may occur over a 24-hour period.³

Erythrocyte Fragility. Reported work is too limited for an established figure. Wirth²³ lists minimum resistance as 0.74–0.78 per cent saline and maximum resistance as 0.58 to 0.66. Kato¹¹ using a micro-method reported initial hemolysis to begin at 0.615 per cent saline and to be complete at 0.48 per cent. Ponder¹⁷ states that when animals are listed in the order of their red cell volumes, they are also arranged in order of the degree of hypotonicity which just causes hemolysis. The goat has the smallest red cell volume (MCV) among the domestic animals and on the basis of Ponder's findings the goat red blood cell should be the most sensitive to hypotonic saline.

Leukocyte Counts, Total and Differential. From the data presented in Table 27, it appears that the normal value for total leukocyte number must be increased by several thousand above that of the cow and sheep. The mean neutrophil count on a percentage basis is greater than either the cow or sheep while mean lymphocyte percentage is lower and the means for the eosinophil and monocyte are comparable to the sheep.

The Myeloid-Erythroid Ratio. Winqvist¹⁰⁴ has reported on the cellular composition of the bone marrow based on sternal puncture of 10 goats.

	%		%
Promyelocytes	0.79 ± 0.15	Neut. bands	8.88 ± 0.88
Neut. myelocytes	2.69 ± 0.49	Neutrophils	9.98 ± 1.47
Eos. myelocytes	0.85 ± 0.19	Eosinophils	1.79 ± 0.34
Neut. metamyelocytes	8.25 ± 1.22	Basophils	0.06 ± 0.03
Eos. metamyelocytes	1.67 ± 0.39		

$$\text{Myeloid total} = 34.95$$

Prorubricytes	1.45 ± 0.18
Rubricytes	30.61 ± 1.48
Metarubricytes	24.27 ± 2.93

Erythroid total = 56.33

Lymphocytes	7.49 ± 2.23
Monocytes	0.02 ± 0.01
Plasma cells	0.11 ± 0.03
Reticulum cells	0.52 ± 0.26
Megakaryocytes	0.00 ± 0.00
Stem cells	0.58 ± 0.14

M:E = 0.69 ± 1.16 (as reported, however, $34.95 \div 56.44 = 0.62$)

THE HORSE

<i>Hot-blooded</i>		<i>Cold-blooded</i>	
<i>Range</i>	<i>Ave.</i>	<i>Range</i>	<i>Ave.</i>
Erythrocytes	7.0-13.0	5.5- 9.5	7.5
Hemoglobin	10.0-18.0	8.0-14.0	11.5
PCV	32.0-55.0	24.0-44.0	35.0
MCV	37.0-50.0	39.0-52.0	44.0
MCH			15.0
MCHC	31.0-35.0	31.0-35.0	33.0
Leukocytes	7.0-14.0	6.0-12.0	8.5
Band	0.0- 2.0	0.0- 2.0	0.5
Neutrophil	30.0-65.0	35.0-75.0	54.0
	N/L = 1:1		N/L = 5:3
Lymphocyte	25.0-70.0	15.0-50.0	35.0
Monocyte	0.5- 7.0	2.0-10.0	5.0
Eosinophil	0.5-11.0	2.0-12.0	5.0
Basophil	0.0- 3.0	0.0- 3.0	0.5
Reticuloocytes	none		none
Sedimentation rate:	Due to marked rouleau formation erythrocyte sedimentation rate is normally high.		
RBC diameter		4.0- 8.0	5.5-5.8
Resistance to hypotonic saline		0.39-0.56	0.45
Thrombocytes		$1-6 \times 10^6$	3.3
Icterus Index		5.0-20	10-15
Specific gravity		1.042-1.060	1.052
M:E ratio		0.94-3.76:1.0	1.64:1.0

For interpretation of the hematology of the horse it is necessary to know whether the animal is classed as "hot" or "cold" blooded. Horses with considerable Arabian ancestry are commonly referred to as "hot-blooded" and these are essentially the Arabians *per se* and the Thoroughbreds.

The pure blooded Arabian is a small horse of unusual endurance. The Thoroughbred is of larger size and was developed by crossing Arabian stallions with English mares. The greater body size enhances its speed and thus the Thoroughbred has become the favorite sports horse.

American horses of Arabian ancestry are the American Saddle Horse, the Morgan Horse, and the Standard Bred Trotter. It is of interest to note that the cow ponies and wild mustangs of the Western United States,

from which the Quarter Horse was developed, are also of Arabian ancestry. The historical background for this is as follows: The Moslems invaded Spain in about 700 A.D. and left in about 1,400 A.D. During this period of occupation, the Arabian horse became well established in Spain and was called the Spanish Barb. The Spaniards brought these horses to America from whence they fell into the hands of American Indians of the western plains. In time great herds of wild horses of Arabian ancestry developed in the western United States.

Erythrocyte Number, PCV, and Hemoglobin. As students of the blood we are interested in the fact that a probable explanation for the endurance and speed of the "hot-blooded" horses may be found in the smaller size and greater number of erythrocytes per unit volume of blood.^{145,151} The smaller size leads to an increase in total surface area of the erythrocyte mass which in turn enhances the rapid exchange of the respiratory gases in the lungs and body tissues.

Neser¹⁵⁴ was of the opinion that the mode of life of the horse is a factor that influences the number of erythrocytes; he reported lower PCV values in idle horses than in horses at work. Neser makes mention that in 6 race horses in full training the red cell counts were always high, *e.g.*, 9.1–11.1 million per cmm. Neser was not aware, however, of differences in erythrocyte number between the breeds. MacLeod and Ponder¹⁵⁰ reported consistently higher levels of hemoglobin and erythrocytes in Thoroughbreds and they considered the difference to be genetic in origin. The MCH is lower in the Thoroughbred^{150,159} due to smaller size of the erythrocyte, but hemoglobin concentration per unit volume of blood is greater because of the larger number of red cells. MacLeod¹⁵¹ observed an occasional nucleated erythrocyte in three Thoroughbreds in prime racing condition; however, no correlation was found between total red cell number and physical condition as reported by Neser.

Irvine¹⁴⁷ has reported a mean hemoglobin value of 11.4 grams per cent among 184 race horses, 3 years old and over, and in training. A subgroup of the best performing horses gave the following: Hb. 12.0, PCV 35, and MCHC 34 as compared to the overall averages of 11.4, 36, and 31.7, respectively. Irvine points to the higher saturation of the erythrocyte with hemoglobin (MCHC) in the best performing horses.

The erythrocyte count of the "hot-blooded" horse will range 2–3 million more per cmm. of blood than in "cold-blooded" horses; mean values being about 9.75 and 7.5, respectively. The hemoglobin concentration is 2 to 4 grams higher per 100 ml. of blood for the "hot" horses; 13.4 grams as compared to a mean of 11.5 grams for "cold" horses. The PCV range is 32–55 for "hot" horses and 24–44 for "cold" horses with means of 42 and 35, respectively.

Influence of Age on Erythrocytes.^{131,134,142,158} In the Thoroughbred,

the erythrocyte number increases up to the third month of life reaching levels as high as 15 million cells per cmm. of blood; at the same time the MCV is declining, dropping from a mean of 33 to 30.4. After the third month of life the erythrocyte number shows a steady decline to the mean adult level of about 10 million and at the same time the MCV increases. The adult red cell on an average is about 10 cubic microns larger than that of weanlings.

Influence of Sex, Pregnancy, Lactation, Barrenness. The stallion of the Arabian¹⁴⁵ or Thoroughbred¹⁴³ shows slightly higher values for erythrocytes and hemoglobin than mares, regardless of whether the latter are pregnant, lactating, or barren. Mares in foal compared with barren mares^{141,144,159} differed very little in their erythrocytic values. However, lactating mares tended to have lower mean values for erythrocytes, PCV and hemoglobin.^{146,159}

Influence of Excitement. Nesser¹⁵⁴ points out that the mechanical state of the circulation, that is pulse rate, is a very important factor influencing the PCV or erythrocyte count in any part of the body; a slow peripheral circulation results in concentration in the capillaries and dilution in the jugular vein. The circulation can be influenced by trifling occurrences in a very excitable animal. Hansen¹⁴¹ verified the statement of Nesser and reported a rise of 1.5 million erythrocytes per cmm. in a Thoroughbred weanling and rise of 4.0 million in a 20-year-old barren Thoroughbred mare after only one minute of excitation. In racing horses, it can be anticipated that erythrocyte counts, PCV and hemoglobin levels will be lowest when the animals are at rest in the stable and highest immediately after the excitement and strenuous exercise of a race. Irvine¹⁴⁷ states that venepuncture by a stranger could cause an increase in erythrocyte number of 10–15 per cent and much more with some conditions of temperament (also, see Chapter 5, p. 213).

Effect of Intestinal Parasites on Erythrocytic Values. Hansen¹⁴² reported on the worm burden of weanlings from two Kentucky farms. The infestations were considered light to moderate and typical or representative of all weanlings in the study. Erythrocyte counts of 11.5 million and hemoglobin levels of 12 grams were reported and thus a significant effect of the parasitism does not appear to have existed. Archer and Poynter¹³⁶ investigated the blood picture in two groups of crossbred ponies; one group was regularly treated with anthelmintics and the other was left to acquire naturally a considerable parasitic burden. A normocytic normochromic anemia, with hemoglobin dropping to as low as 5–6 grams per 100 ml. of blood, developed in association with erythrocytic hypoplasia in the bone marrow.

Erythrocyte Diameter. The reports in the literature on erythrocyte diameter probably relate specifically to the "cold blooded" horse. The range in size of individual cells is 4 to 8 microns¹⁵⁴ with a mean size of

5.6–6.0.^{10,18,19,137,154} One author gives the mean diameter as 5.3 microns.⁹⁰

Reticulocytes. The erythrocytes of the equine are released from the bone marrow in a mature state and, therefore, reticulocytes are not found in peripheral blood in health and only to a very limited extent following severe hemorrhage. Therefore, polychromatophilia and reticulocytosis are not characteristic of the response to blood loss or hemolytic anemia in the equine (see Appendix, Case 13, p. 347).

Sedimentation of Erythrocytes. Erythrocyte rouleau formation is very prominent in equine blood and this phenomenon is associated with a rapid sedimentation rate. Since rouleau is so pronounced in the equine, Knisely *et al.*¹⁴⁹ were interested in determining whether agglutination of erythrocytes occurs within the vascular system of normal horses. They made microscopic observations on the *in vivo* capillary blood of the eye of nine carefully selected healthy horses and reported that no evidence of agglutination was found. However, trauma to one healthy horse caused microscopically observed precipitation and agglutination of blood cells passing through vessels in and near the site of injury, and more severe trauma to another equine resulted in the formation of masses of agglutinated erythrocytes which were sufficiently hard and rigid to remain intact while circulating.

Although horse erythrocytes characteristically settle rapidly, the rate of settling is influenced to a considerable degree by the relative volumes of cells and plasma. The PCV range in the “cold” horse is 24–44, while in the “hot” horse it is 32–55. Thus, in general, faster settling may be anticipated in the blood of the former.

Hammersland *et al.*¹⁴⁰ using the Cutler method found the average ESR of 60 normal horses to be 18 mm. at the end of 1 hour with a range of 0 to 30 mm. He found the sedimentation rate to be elevated in equine infectious anemia but this increase in rate was later shown by Gilman¹³⁹ to be correlated with the degree of reduction of erythrocyte volume due to the anemia. Van Zijl,¹⁶⁰ whose work is cited by Gilman, used the Westergren procedure and calculated the mean sedimentation rate per 10 minutes, corrected to a PCV of 30, to be 21.9 ± 4.58 . Since Van Zijl selected PCV 30 as representative of the average normal PCV for his horses, it can be assumed that he was working with “cold blooded” equines. Gilman,¹³⁹ on the other hand, used a PCV of 42 for correction purposes and from this it can be inferred that he was working with “hot blooded” equines. Using the method of Van Zijl to determine the mean sedimentation rate per 10 minutes (SR/10) and correcting to PCV 42 by means of a correction chart prepared with the aid of Van Zijl’s data, Gilman found the average normal SR/10 to be 15 mm. with a range of 7–23 mm. encompassing 97 per cent of the samples. Van Zijl’s formula for SR/10 is

$$SR/10 = \frac{\frac{a}{3} + \frac{b}{6}}{2}$$

a = 30 minute reading; b = 60 minute reading

Coffin⁵ reports the ranges for erythrocyte sedimentation of normal equine blood in the Wintrobe hematocrit tube to be 2–12 mm. in 10 minutes and 15–38 mm. in 20 minutes.

Erythrocyte Fragility. Limited reports indicate the percentage saline for initial hemolysis and complete hemolysis to be, respectively; 0.51 and 0.39,¹ 0.56 and 0.30,¹⁹ 0.55 and 0.40.¹⁵⁷

Leukocyte Counts, Total and Differential. (Tables 28 and 29) Total counts range from 6–12 thousand per cmm. of blood for “cold blooded” and from 7–14 thousand for “hot blooded” horses. The mean counts are 8–9 thousand and 9.5–10.5 thousand, respectively. The influence of age has been studied^{142,158} in the Thoroughbred. During the first month of life, the total count is at its lowest with a range of 5–11 thousand and a mean of 8 thousand. The total count increases so that during the third through the sixth month of life the normal range is almost doubled and the mean count reaches 14 to 15 thousand. In weanlings the total count begins to fall slightly, but counts as high as 18 thousand may be anticipated and the mean count is 13–14 thousand. Among mature Thoroughbreds, stallions¹⁴³ have a lower total leukocyte count than mares,^{141,144,146} the counts in stallions being similar to the range and mean stated or the “cold blooded” horse.

MacLeod¹⁵⁰ called attention to the fact that in Thoroughbreds the neutrophil-lymphocyte ratio approximates 1:1 as compared to a 5:3 ratio in cold horses. During the first 2 months of life of the Thoroughbred the ratio is 6:4¹⁵⁸ followed by a reduction in neutrophils and an increase in lymphocytes reaching the 1:1 ratio in the weanling stage of life. Limited observations¹⁴⁵ in mature Arabian horses indicates a slightly higher neutrophil percentage than in the mature Thoroughbreds.

The Myeloid-Erythroid Ratio. Studies on cellular content of the bone marrow of equines are very limited. Calhoun^{105,138} and Archer¹³⁴ have reviewed the literature and presented observations of their own on differential cell counts and have supplied photographs of various cell types in the marrow (Table 30).

Archer used 12 mongrel ponies representing crosses between one or more breeds. Marrow aspirations were made from the tuber coxae, midway between its two tuberosities. A local anesthetic was applied to the skin and periosteum. The needle was introduced into the bone by a steady rotary screwing motion, directing the point at the hip joint of the opposite side. In foals only ⅝-inch penetration was required, but in animals 10 years of age or older, the needle had to be inserted 2 inches

Table 28. Hematology of the Thoroughbred Horse¹

Lit. Ref.	Class	No. ²	RBC ¹ 10 ⁶	Hb. gm. %	PCV	MCV	MCHC	MCH ³	WBC 10 ³	Differential Leukocyte Count in %			
										Neut.	Lymph.	Mono.	Eos.
158	Foals 1-4 wks.	10/10	9-14 (11)	11-14 (12)	33-44 (36)	27-38 (33)	32-35 (33)	10.9	4.8-11.3 (8.36)	38-81 (59)	19-62 (39)	0-3.5 (1.5)	0-1.0 (0.29)
	Foals 5-8 wks.	14/9	9-15 (12)	11-14 (12.6)	33-44 (38)	27-36 (31.5)	31-36 (33)	10.5	6.3-15.7 (10.61)	42-74 (59)	24-55 (39)	0-4 (1.9)	0-1.5 (0.4)
	Foals 9-12 wks.	19/14	10-15 (12.8)	11-16 (12.9)	32-49 (39)	26-34 (30.4)	30-36 (33)	10.1	9.4-21.6 (13.64)	33-63 (49)	35-64 (48)	0-4 (1.9)	0-9.5 (1.12)
142	Foals 13-19 wks.	18/17	9-15 (11.8)	10-16 (12.2)	31-47 (36)	28-35 (30.8)	29-43 (34)	10.3	9.3-19.2 (13.67)	26-71 (53)	27-74 (42)	0-7.5 (2.4)	0-7.0 (2.4)
	Foals 20-26 wks.	11/9	9-14 (11)	10-14 (11.7)	29-42 (35)	25-37 (31.7)	30-37 (33)	10.6	9.9-20.5 (14.53)	30-82 (53)	15-67 (41)	0-14 (2.9)	0-10 (3.0)
	Weanlings	70	9-13 (11)	10-14 (12)	30-42 (37)	30-36 (32.7)	30-35 (33)	10.9	7.6-18.6 (13.64)	21-74 (45)	23-79 (50)	0.5-7.6 (2.7)	0-1.0 (0.1)

141	Mares in Foal	65	9-13 (10.5)	13-17 (14.9)	38-51 (45)	38-48 (42.9)	31-36 (33)	14.2	6.8-13.5 (9.97)	30-66 (49)	24-65 (44)	0.5-6.5 (2.7)	0.5-9.5 (3.7)	0-3.5 (0.7)
146	Non-lact. mares	17	7-11 (9.7)	11-16 (13.5)	37-49 (42)	37-55 (43.7)	29-35 (32)	13.9	7.3-14.0 (10.8)	25-61 (44)	35-72 (50)	0-4.5 (1.3)	0.5-8.0 (3.9)	0-2 (0.5)
	Lactating mares	17	7-10 (8.3)	10-14 (11.9)	32-45 (37)	42-48 (43.8)	31-34 (33)	14.3	7.4-11.7 (9.58)	23-54 (42)	36-72 (51)	0-4 (1.5)	2.5-11 (5.0)	0-0.5 (0.5)
144	Barren mares	70	8-11 (9.9)	12-16 (13.8)	35-49 (43)	37-49 (43)	29-35 (32)	13.9	7.1-14.1 (10.37)	25-63 (48)	30-72 (46)	0.5-6.0 (1.7)	0.5-11.5 (4.6)	0.5-2.5 (0.5)
143	Stallions	36	8-13 (10.8)	12-18 (14.7)	38-59 (47)	39-46 (43)	30-34 (31)	13.6	5.1-11.9 (8.27)	35-65 (51)	30-58 (44)	0.5-7.0 (2.0)	0.5-7.0 (4.0)	0-2.0 (0.5)
<i>The Arabian Horse</i>														
145	Barren mares	5	7-11 (9.6)	10-15 (13)	30-45 (40)	40-43 (41)	32-34 (33)	13.5	6.1-11.8 (9.42)	52-58 (56)	36-40 (39)	0-3.5 (2.2)	2.5-4.5 (3.5)	— (0.5)
	Mares in foal	4	8-10 (9.2)	12-13 (12.4)	33-43 (37)	40-42 (41)	31-35 (34)	13.5	6.5-9.6 (8.11)	37-52 (46)	41-57 (48)	1-1.5 (1.2)	2.5-8.0 (4.9)	0
	Stallions	7	9-11 (10)	12-15 (13)	35-46 (39)	38-42 (39)	33-34 (33.5)	13	6.4-10.9 (8.65)	43-71 (58)	28-51 (38)	0-3.5 (2.1)	1-3 (2.3)	0

¹ For data on English Thoroughbred Horse see the report by Archer¹³⁵.

² No. foals for erythrocytic series/no. foals for leukocytic series.

³ From author's mean RBC and Hb. values.

Table 29. Hematology of the Cold-Blooded Horse

Literature Reference	Type	No.	RBC 10 ⁶	Hb. gm. %	PCV	MCV	MCHC	WBC 10 ³	Differential Leukocyte Count in %					
									Band	Neut.	Lymph.	Mono.	Eos.	Bas.
Moore ¹⁵²	Mixed	7	7.14-9.93 (7.94)	—	—	—	—	4.3-6.8 (5.62)	—	51-69 (59)	24-36 (30)	2-10 (6)	3-6 (4)	0.1-3 (1)
Wells ²²	—	2	7.894	—	—	—	—	8.60	—	—	—	—	—	—
Burnett ⁴	Stallion	—	7-10	—	—	—	—	8.4-11.0	—	—	—	—	—	—
	Gelding	—	5.5-9.0	—	—	—	—	6.9-9.4	—	—	—	—	—	—
	Mare	—	5.5-7.5	—	—	—	—	6.5-9.0	—	—	—	—	—	—
Neser ¹⁵⁴	Mixed	26	5.2-11.45 (7.76)	—	23-48.5 (32.9)	— 42.9*	—	<i>see footnote</i>	—	52.0	39.0	4.0	4.0	1.0
Kohanawa ¹²	—	—	7.20	—	—	—	—	8.06	—	54.2	38.1	2.6	4.7	0.4
Scarborough ¹⁹	—	—	6.0-8.5 (7.8)	—	—	—	—	6-12.0 (9.26)	—	50-65 (56.8)	20-40 (30.4)	2-12 (8.5)	1-5 (3.7)	0-1 (0.46)
Hammersland ¹⁴⁰	—	60	5.84	—	—	—	—	8.24	—	52.0	42.0	4.0	1.0	1.0
Holman ¹⁵⁷	Clydesdale	36	5.7-8.8 (6.95)	8.1-11.0 (9.55)	24-34 (27)	— 38.8*	— 35.3*	6.6-12.4 (8.8)	0.5-8 (3)	34-78 (52)	13-56 (31.5)	1-8 (5)	1-28 (8)	0-3 (0.5)
Morris ¹⁵³	Army	10	7-9.49 (7.3)	—	—	—	—	5.7-10.8 (8.9)	0.5	55.9	35.0	3.4	4.3	0.8
Trum ¹⁵⁹	Percherons	11	5.7-9.55 (7.39)	10.1-14.5 (11.67)	—	—	—	6-10.5 (8.01)	—	36-74 (52.2)	20-59 (41.1)	0-5 (1.5)	2-13 (4.7)	—

* Calculated from the author's data.

¹ Neser presents considerable data on total leukocyte counts but it is difficult to obtain ranges and means.

or more. Suction was applied by means of a 20 ml. syringe with a glass plunger and about $\frac{1}{2}$ ml. of marrow was withdrawn.

Calhoun employed 7 old horses, "cold blooded" types and apparently free from disease as far as could be determined by general appearance and blood determinations. The rib was chosen for source of the marrow aspiration. Under local anesthesia a 3/32 inch drill was used to penetrate the bone at midpoint between the anterior and posterior borders. One

Table 30. Cellular Composition of the Bone Marrow of the Horse

<i>Cell Types</i>	<i>Data by Archer (12 ponies)¹³⁴</i>		<i>Data by Calhoun (7 horses)¹³⁸</i>	
	<i>Range %</i>	<i>Mean %</i>	<i>Range %</i>	<i>Mean %</i>
Myeloblast	0.0 - 0.66	0.237	—	—
Progranulocyte	0.14- 0.99	0.502	0.0 -5.0	1.83
Myelocyte neut.	11.40-28.60	17.94	26.2-56.0	38.06
Myelocyte eos.	0.69- 2.35	1.43	0.4- 3.6	2.34
Metamyelocyte neut.	17.90-29.40	21.80	—	—
Metamyelocyte eos.	1.05- 6.95	3.46	—	—
Metamyelocyte bas.	0.14- 5.39	0.99	—	—
Neutrophil	7.83-25.30	14.70	1.8-20.2	13.31
Eosinophil	0.52- 5.95	2.63	0.2- 1.2	0.60
Basophil	0.0 - 0.33	0.05	0.0- 1.0	0.60
Total myeloid		63.73		56.74
Rubriblast	0.0 - 0.87	0.34	0.4- 3.4	1.6*
Prorubricyte	0.16- 4.35	1.88	—	—
Rubricyte	2.21-13.00	6.34	8.0-32.0	20.94
Metarubricyte	4.20-39.20	17.60	5.0-24.2	13.71
Total erythroid		26.16		34.66
Monocyte	—	—	1.2- 4.8	2.46
Lymphocyte	2.46-20.90	9.71	2.0- 5.6	3.91
Plasma cell	0.0 - 1.66	0.50	0.0- 0.8	0.63
M:E	1.1 -10.20	2.43	0.9- 3.76	1.64

* Calhoun called this group "stem cell" and did not include it in the total erythroid cell count.

ml. or less of marrow was removed by use of trocar needle and 10 ml. syringe.

Thrombocytes. In 36 Clydesdale horses the range was found to be 90,000-410,000 with a mean of 210,000 per cmm. blood.¹⁵⁷ Other reports have given counts as low as 73,000¹¹⁹ and as high as 560,000²¹ with means of 330 to 350 thousand. The erythrocyte:platelet ratio is about 32:1 in the horse. In the English Thoroughbred horse thrombocyte counts were 90,000 to 170,000 per cmm. of blood.¹⁴⁷

Icterus Index. The color of normal equine plasma is of a more intense yellow than in any of the other domestic animals, average icterus index reading being 10-15 units. Ramsay¹⁵⁵ determined the bilirubin concentration in 77 plasma samples representing 51 horses. The mean result was 1.57 mg. per 100 ml., S.D. 0.86 mg. The lowest concentration was 0.48 mg. per 100 ml. and five samples contained over 3 mg. per 100 ml. of plasma. Comparing equine with human plasma, Ramsay

found the former to contain 3 times as much bilirubin as the latter. Jennings and Mulligan¹⁴⁸ in 30 horses gave a range of 0.6–1.7 mg. of bilirubin per 100 ml., mean 0.98 mg. per cent.

Another interesting observation was that fasting for 48 hours causes a marked increase in plasma bilirubin. An extreme example was reported to be in a horse with 1.96 mg. per 100 ml. which rose to 5.1 mg. while fasting and returned to 1.86 mg. in 4 days after the fast was broken. This observation indicates that icterus index readings in the horse must be interpreted with considerable caution. The blood plasma of the horse on green feed contains the yellow pigment carotin^{15a}.

Specific Gravity. The range in the blood of 36 Clydesdale horses¹⁵⁷ was found to be 1.047–1.060 with a mean of 1.053. In Scarborough's¹⁹ literature survey through 1926, 14 animals reported upon had a range of 1.042–1.054 and a mean of 1.045. Reichert¹⁸ records the mean specific gravity of equine blood as 1.060.

BLOOD VALUES OF THE DONKEY AND MULE

A limited number of observations has been made on the hematology of the donkey in South Africa and the burro of southwestern United States. Studies on the blood of mules have been made in South Africa and in England. These data are presented in Table 31.

The Erythrocytes. Nesor¹⁵⁴ reported the diameter of the red cell in the donkey to have a range of 4.8–7.2 with a mean of 6.2 microns and for the mule to have a range of 4.8–8.8 with a mean of 6.23. However, the MCV determined from Nesor's data reveals the mule erythrocyte to have a mean value of 43.5 cubic microns whereas that of the donkey is 57.8. The mean value for MCV of the erythrocyte of the burro for both male and female as determined from the data of Wilding¹⁶¹ was 52.1 cubic microns. The mean erythrocyte count per cmm. blood for the donkey and burrow fell between 6.4 and 7.1 millions while that of the mule ranged from about 7.4 to 8.0 millions. These data indicate that erythrocyte counts of mules and donkeys are similar to those of the "cold blooded" horse but that the cell is somewhat larger. The larger erythrocyte leads to a generally higher mean PCV than is observed in "cold blooded" horses but considerably lower than is commonly found in the "hot blooded" horses. The hemoglobin level of the burrow ranged from 8.0–16.7 grams per 100 ml. with means of 11.6 to 12.1 grams. Erythrocyte sedimentation was conducted on the blood of the burrow by Wintrobe method and the reading made at the end of 1 hour. For males, the range was 21–63 mm. with a mean of 54.3 mm.; for females, the range was 4–67 mm. with a mean of 47.5 mm.

The Leukocytes. The mean total leukocyte count for donkeys was 12,600 to 14,400. This is considerably higher than means for mature

Table 31. Hematology of Donkeys and Mules

Lit. Ref.	Class	No.	RBC 10^6	Hb. gm. %	PCV	MCV ¹	MCH ¹	MCHC ¹	WBC 10^3	Differential Leukocyte Count in %				
										Neut.	Lymph.	Mono. ¹	Eos.	Bas.
Neser ¹⁵⁴	Donkey ²	14/32	4.6-8.5 (6.4)	—	30-48 (37)	— (37.8)	—	—	11.5-21.9 (14.4)	15-57 (34)	33-71 (53)	2-15 (4)	1-14 (8)	0-1 (1)
Wilding ¹⁶¹	Burro ³ (male)	16	5.65-8.58 (6.91)	9.3-15.5 (11.61)	31-45 (36)	— (52.1)	— (16.8)	— (32.2)	7.0-17.7 (12.6)	26-65 (43)	18-64 (42)	1-13.5 (6)	0-18 (7.5)	—
	Burro ³ (female)	12	4.95-10.27 (7.1)	8.0-16.7 (12.11)	26-51 (37)	— (52.1)	— (17.0)	— (32.7)	9.3-28.5 (14.4)	28-64 (43)	24-59 (42)	0-11.5 (5.5)	0.5-26 (8)	—
Neser ¹⁵⁴	Mule ²	15/28	4.86-10.60 (8.04)	—	30-42 (35)	— (43.5)	—	—	9.0-18.3 (12.9)	32-70 (49)	22-60 (41)	1-5 (3)	2-13 (6)	0-2 (1)
Morris ¹⁵³	Mule ⁴	20	6.17-9.93 (7.37)	—	—	—	—	—	6.0-10.75 (8.94)	33-60 (43)	36-60 (47)	2-7 (4)	2-11 (5)	0-1 (0.6)

¹ Estimated from the authors means.

² South African mules and donkeys.

³ Southwestern U.S.A. burros.

⁴ English army mules.

"hot" or "cold blooded" horses. In the case of mules, Nesor in South Africa gave a mean of 12,900 but Morris in England reported 8,940. The differential leukocyte count of both the donkey and the mule is similar to "hot blooded" horses in that the neutrophil:lymphocyte ratio approximates 1:1. This was true with the exception of the data of Nesor in South Africa in which the lymphocytes exceeded the neutrophils, giving a ratio of 3:5.

Other Data. Sastry¹⁵⁶ in India compared 10 normal mules with 10 horses all kept at an elevation of 7,500 feet. The original article was not available but an abstract stated the following: The blood of the mules was characterized by slightly larger erythrocytes; high PCV, MCV, and MCH; longer prothrombin time; lower icterus index and bilirubin; lower calcium and phosphorus, and higher gamma globulin. The authors confirmed the absence of reticulocytes from the blood of the horse.

THE PIG

<i>Erythrocytic Series</i>			<i>Leukocytic Series</i>		
	<i>Range</i>	<i>Ave.</i>		<i>Range</i>	<i>Ave.</i>
Erythrocytes	5.0- 8.0	6.5	Leukocytes	11-22,000	16,000
Hemoglobin	10.0-16.0	13.0	Band neut.	0-4	1.0
PCV	32-50	42.0	Neutrophil	28-47	37.0
MCV	50-68	63.0	Lymphocyte	39-62	53.0
MCH	17.0-23.0	20.0	Monocyte	2-10	5.0
MCHC	30.0-34.0	32.0	Eosinophil	0.5-11	3.5
Reticulocytes	0.0-1.0	0.4	Basophil	0-2	0.5
Sedimentation rate/hour	variable		<i>Other Data</i>		
RBC diameter	4.0- 8.0	6.0	Thrombocytes	520,000	± 195,000
Resistance to hypotonic saline:			Icterus index—less than	5 units	
Initial hemolysis		0.70	Specific gravity	1.055-1.061	1.059
Complete hemolysis		0.45			
M:E ratio = 1.77 ± 0.52					

THE PROBLEM OF ADEQUATE IRON FOR SUCKLING PIGS

Newborn pigs have mean hemoglobin levels of 10-12 grams per 100 ml. of blood and the mean total iron content of the body is about 50 mg.¹⁷⁹ Less than one-tenth of this iron is in reserve and available for new blood formation. Milk is notoriously low in iron and the iron content of sow's milk has been reported to be only 0.009 per cent.¹⁷⁵

Suckling pigs grow very rapidly; their weight is doubled by the end of the first week of life and a gain of 4 times the birth weight can be expected by the end of the third or fourth week. It has been estimated¹⁷⁹ that the baby pig will obtain about 6 mg. of iron from milk during the first week and a total of about 23 mg. in the first 3 weeks of life. However, the need for iron to supply hemoglobin for the expanding blood volume approaches 7 mg. per day. A physiologic drop in hemoglobin concentra-

tion occurs during the first week of life as the body doubles in size. A 25–30 per cent reduction in the hemoglobin level is to be anticipated. Unless a source of iron is available, the pigs become severely anemic during the next 2–3 weeks, leading to death, stunted pigs, and/or lowered resistance to disease. Spontaneous recovery begins about the fifth or sixth week of life when the pigs begin to take nourishment in addition to milk.

Pigs raised out-of-doors but on concrete are less anemic than pigs raised indoors.¹⁶⁵ For awhile it was thought that sunlight and exercise¹⁷³ were the required factors for hemoglobin production but soon, thereafter, it was shown that contact with soil, early in life of the pig, was all important in the prevention of anemia.^{164,166,168,170,171,180} Soil may contain as much as 1.5 per cent iron¹⁷⁹ and pigs begin to root and eat dirt about the third or fourth day.¹⁷¹ Washings of the gut were shown to contain as much as 500 mg. of iron.¹⁷⁹ If, due to inclement weather, it is necessary to raise the pigs indoors on concrete floors, it is important to bring soil into the farrowing house.

Supplementing the ration of the sow with iron during pregnancy and lactation may lead to a slight increase in the iron content of the milk¹⁷⁹ but is not sufficient to prevent the development of anemia in the baby pigs.^{165,169,176} Iron may be supplied to suckling pigs by the daily application of a saturated solution of iron sulfate to the teats of the sow or by daily dosing pigs during the second week of life with 30 mg. of iron pyrophosphate.¹⁶⁷ Both these methods are tedious and, therefore, a single treatment method was sought. A single intramuscular injection of 100 mg. of iron in the form of an iron-dextran complex, administered on the fourth day of life, was shown to be an effective means for preventing anemia in baby pigs.^{162,163,172,174,178}

A review of the literature on anemia in young pigs has been presented by Seamer.¹⁷⁷

THE HEMATOLOGY OF YOUNG PIGS

Table 32 summarizes the data on a single litter of 9 pigs, 5 males and 4 females, bled periodically during the early days of life. This litter was farrowed and kept inside on concrete for the first 10 days of life and then transferred outside on soil.

Mean weights reveal a near doubling in 1 week and a four-fold increase by the third week of life. The mean hemoglobin concentration fell 33.3 per cent during the first 10 days but returned to the birth level during the second 10 days when the pigs had access to a natural source of iron in soil. The fall in erythrocyte number by 34 per cent and the PCV by 31 per cent paralleled the reduction in hemoglobin. MCV, MCH, and MCHC did not change significantly during the first 10 days of life but

Table 32. Influence of Age and Husbandry on the Hematology of Young Duroc-Jersey Pigs. Ranges and Means for a Single Litter of 5 Males and 4 Females. Pigs Kept on Concrete Until 10 Days of Age and then Placed on Soil¹

Age Days	Value	Wt. in lbs.	RBC 10^6	Hb. gm. %	PCV	MCV	MCHC	MCH	Retic %	Nuc. RBC	Sed. Rate	WBC 10^3 (**)	Differential Leukocyte Count in %				
													Band	Neut.	Lymph.	Mono.	Eos. Bas.
1	Min.	1.7	4.3	8.4	27.0	57	28.9	18.0	4.5	0.5	0	7.6	1.0	64.5	16.0	0.5	0
	Max.	3.3	6.4	12.3	42.5	71	31.3	21.0	10.0	4.0	4	15.3	7.0	75.5	31.0	7.5	2.0
	Ave.	(2.4)	(5.3)	(10.5)	(35)	(67)	(30.5)	(20)	(6.7)	(2)	(2)	(11.5)	(3.6)	(71)	(20)	(4.7)	(0.9)
3	Min.	2.4	3.3	7.8	26.5	70	29.1	21.0	6.9	7	2	6.3	1.0	38.0	23.5	6.0	0
	Max.	4.0	5.2	11.0	36.5	81	30.3	24.0	16.6	57	12	13.4	5.5	61.5	54.0	9.5	1.5
	Ave.	(3.2)	(4.5)	(9.8)	(33)	(73)	(29.5)	(22)	(12.0)	(17)	(5)	(9.4)	(3.3)	(51)	(37.6)	(6.8)	(0.8)
6	Min.	3.5	3.4	6.4	22.0	60	26.4	17.0	4.5	5	12	7.4	1.0	33.0	32.5	2.0	0
	Max.	5.0	4.7	9.4	31.0	74	30.9	23.0	13.0	54	33	10.5	3.3	60.5	55	10.5	1.0
	Ave.	(4.5)	(4.0)	(8.0)	(26.7)	(67)	(29.1)	(20)	(7.7)	(14)	(22.6)	(8.2)	(2)	(45.4)	(45.3)	(4.9)	(0.3)
10	Min.	5.2	2.1	4.2	15.0	62	29.0	19.0	6.0	3	1	5.6	0	8.0	36.5	1.0	0
	Max.	7.1	4.3	8.7	20.0	78	31.0	24.0	12.0	30	35	19.1	2.0	51.0	82.0	10.0	0.5
	Ave.	(6.4)	(3.5)	(7.0)	(24)	(68)	(29.6)	(20)	(10)	(11)	(12)	(10.9)	(1)	(27)	(64)	(7)	(0.1)
20	Min.	8.5	4.4	9.0	35.5	70	26.0	19.0	9.0	1	0	6.2	0	13.5	55.0	2.0	0
	Max.	11.5	5.3	11.2	40.5	82	29.0	23.0	13.0	25	1	10.5	3.5	39.5	82.0	7.0	2.0
	Ave.	(10.5)	(4.9)	(10.2)	(37)	(76)	(27.6)	(21)	(10.6)	(11.5)	(0)	(7.7)	(1.4)	(25.7)	(66.8)	(4.3)	(0.8)
36	Min.	—	5.9	11.3	37.0	62	28.0	18.8	1.6	0	0	12.7	0	28.0	40.0	3.0	3.5
	Max.	—	6.8	13.3	44.0	68	32.0	20.0	6.8	1	2	20.9	5.0	43.0	68.0	10.5	14.0
	Ave.	—	(6.2)	(12.1)	(39.7)	(64)	(30.5)	(19.4)	(3.0)	(0.5)	(0.5)	(16.3)	(1.8)	(33)	(52)	(6)	(7)

** Corrected for nucleated red cells.

¹ These data were developed in cooperation with Dr. Otto Straub.

during the second 10 day period, the MCV increased from about 68 $c\mu$. to 76 $c\mu$. and the MCHC decreased from 29.7 per cent to 27.6 per cent, while MCH, or weight of the hemoglobin in the red cell, remained about the same.

Intense erythrogenesis during the first 3 weeks of life was reflected in high levels of reticulocytes, nucleated erythrocytes, Howell-Jolly bodies and polychromatophilia in peripheral blood. Crenation of erythrocytes was a constant feature, erythrocyte rouleau formation was variable and after the sixth day slight poikilocytosis was noted.

The sedimentation of erythrocytes was nil or slight at birth but increased reaching a peak at the end of the first week of life and then tapered to zero by the end of the third week. Diphasic reactions were consistently observed, being a reflection of the high level of reticulocytes in peripheral blood.

The icterus index was uniformly 5 units or less and the plasma often appeared opalescent or cloudy.

The total leukocyte count must be corrected for nucleated red cells since the number of the latter may go above 50 per 100 WBC. At 1 day of age the total leukocyte number was slightly higher than during the succeeding 3 weeks. The most significant change with growth was in the differential count. At birth the neutrophils approach 70 per cent and the lymphocytes 20 per cent. The neutrophils decreased quickly, while the lymphocytes increased so that by the tenth day, the percentages of the two cell types were reversed.

PORCINE HEMATOLOGY IN GENERAL (Table 33)

Erythrocyte Number. The number varies with age and husbandry during the suckling and growing period.^{170,184} At birth, a range of 4-7 million may be anticipated, with a mean of 5-6 million. A 30 per cent reduction in count by the tenth day of life occurs as a result of rapid growth and lack of a significant body reserve of iron. If the pigs are in contact with a natural source of iron as in soil, the erythrocyte count will increase, and by 2 months of age the adult levels of 5-8 million with means falling between 6-7 million are approximated.^{19,192}

Erythrocyte Size. The red cell of the new born is about 10 cubic microns larger than that of the mature pig.¹⁷⁴ The diameter of the red cell in the mature pig ranges from 4-8 microns with a mean of about 6.0 μ .

Reticulocytes. Reticulocytes, nucleated erythrocytes and polychromatophilia are very prominent in the peripheral blood of suckling pigs. When the erythrocyte count approaches the adult level, the nucleated red cells disappear and the reticulocyte percentage falls to less than 1.0 per cent. Occasionally polychromatophilic erythrocytes are found in peripheral blood of mature swine.

Table 33. *The Hematology of Duroc-Jersey Swine as Influenced by Age, Sex, Castration, Pregnancy and Parturition*¹

Classification	No.	Value	RBC 10 ⁶	Hb, gm. %	PCV	MCV	MCHC	Sed. Rate	WBC 10 ³	Differential Leukocyte Count in %								
										Myelo.	Mdta.	Band	Neut.	Lymph.	Mono.	Eos.	Bas.	
Both sexes 3½ to 4 months	10	Min.	6.4	11.5	38	53	28	0	18.9				1.0	17	46	1.0	0.5	0.0
		Max.	8.0	13.3	44	61	31	6	33.8				3.0	42	77	8.0	8.5	1.5
		Ave.	7.1	12.0	40	57	30	2.6	26.9	0.0	0.0		2.0	27	63	5.0	2.5	0.5
Castrated males 3 to 6 months.	16	Min.	6.0	9.8	31	54	28	0	11.1				0.5	17	44	2.0	0.0	0.0
		Max.	8.0	13.0	44	68	32	25	28.3				4.5	45	73	10.0	10.0	1.0
		Ave.	7.0	11.7	39	59	30	5	19.5	0.0	0.0		2.0	29	60	6.0	2.5	0.5
Males 6 to 12 months	9	Min.	6.3	11.5	37	55	29.5	1	13.4				0.0	30	51	4.0	0.5	0.0
		Max.	8.6	13.5	44	68	33	14	25.3				2.5	41	61	9.0	4.5	1.0
		Ave.	7.0	12.4	41	59	31	5	18.9	0.0	0.0		1.5	33	57	6.0	2.2	0.3
Males 1 year and older	8	Min.	5.8	12.8	41	62	30	0.5	10.0				0.0	11	36	5.0	2.0	0.0
		Max.	7.5	15.3	50	72	33	31	17.4				2.0	49	76	12.0	5.5	3.0
		Ave.	6.7	14.1	45	66	31	13	13.3	0.0	0.0		0.6	32	55	8.0	3.5	0.9
Females 6 to 12 months; not pregnant	10	Min.	5.4	10.4	36	53	30	5	14.5				0.5	19	53	3.5	0.0	0.0
		Max.	7.9	13.8	46	67	34	27	21.6				3.5	37	67	10.5	5.5	2.0
		Ave.	7.0	12.9	41	59	32	15	15.3	0.0	0.0		1.2	33	57	5.5	2.7	0.6
Females 1 year and over; not pregnant	9	Min.	4.7	9.6	31	56	30	24	11.6				0.0	28	38	0.0	0.5	0.0
		Max.	7.7	14.3	48	69	33	55	21.0				2.0	42	61	9.0	10.0	1.5
		Ave.	6.0	12.1	38	64	31	26	16.4	0.0	0.0		0.7	36	54	5.0	4.0	0.3

Females 1 year and over; pregnant 3 to 8 weeks	20	Min. Max. Ave.	5.6 8.0 6.9	11.5 14.7 13.3	37 48 43	58 68 63	30 32 31	1 30 7	11.3 22.3 16.3	0.0 0.0 0.0	0.0 2.5 1.0	31 48 37	39 61 51	2.5 11.0 6.0	1.0 12.0 4.0	0.0 2.0 1.0
Females 1 year and over; pregnant 2½ to 3½ months	38	Min. Max. Ave.	5.1 8.0 6.4	11.2 15.3 12.8	35 50 42	59 69 65	29 33 31	3 53 20	9.8 20.9 14.4	0.0 0.5 0.1	0.0 4.5 1.1	23 58 35	30 68 55	0.5 12.0 5.0	0.0 9.0 3.0	0.0 2.0 0.8
Females 2 weeks or less before parturition	14	Min. Max. Ave.	4.9 6.3 5.7	11.0 14.5 12.6	34 46 40	63 75 70	30 33 31.6	0 47 21	11.5 21.9 15.6	0.0 0.0 0.0	0.0 3.5 1.7	25 55 39	34 57 52	1.5 9.0 6.0	0.0 2.5 0.7	0.0 1.5 0.6
Females 1 to 6 hours postpartum	5	Min. Max. Ave.	4.9 6.5 5.7	11.2 12.8 12.1	35 42 38	57 73 66	31 32 31.8	21 45 33	15.1 19.2 17.3	0.0 1.0 0.2	0.0 5.5 2.8	43 67 52	17 48 33	0.5 9.0 5.0	0.5 11.5 7.0	0.0 0.0 0.0
Females 10 to 24 hours postpartum	8	Min. Max. Ave.	4.5 7.3 5.8	10.0 14.5 12.0	30 46 37	57 70 63	31 33 32	10 54 47	7.0 17.2 10.3	0.0 4.0 0.5	0.0 6.0 1.8	8 45 30	34 72 46	2.5 11.0 7.0	1.0 6.0 4.0	0.0 1.5 0.7
Females 2 to 10 days postpartum	13	Min. Max. Ave.	4.5 6.9 5.5	9.8 15.1 12.7	30 47 39	59 71 66	31 34 32	0 28 12	7.8 21.0 15.0	0.0 0.0 0.0	0.0 7.5 3.0	4 58 43	29 57 40	2.5 30.0 9.0	0.5 9.5 4.0	0.0 3.0 1.0
Females 15 to 49 days postpartum	10	Min. Max. Ave.	2.4 6.0 4.9	5.1 12.3 10.4	15 42 32	61 79 66	29 35 32	3 55 35	8.8 24.4 18.7	0.0 3.0 0.6	0.5 14.0 4.0	36 59 46	31 52 37	2.0 11.5 6.0	2.0 10.0 5.0	0.0 3.5 1.4

¹ These data were developed in cooperation with Dr. Otto Straub.

Blood Volume and Volume Per Cent Erythrocytes (PCV). Total blood volume was shown to decrease from 95 ml. per kilogram in young pigs to 56 ml. per kilogram in pigs weighing over 200 pounds of the Chester White breed.¹⁸² In the Hampshire breed¹⁸⁶ the blood volume was 74 ml. per kg. in pigs averaging 10 pounds and this decreased to 46 ml. per kg. in pigs weighing over 300 pounds. As the pig grows, the plasma volume per unit volume of body weight decreases more rapidly than the red cell volume and hence the larger pigs have a higher volume per cent of erythrocytes.¹⁸² In anemic swine, the reduction of red cell volume is not compensated by a comparable increase in plasma volume. This results in a slightly lower total blood volume in the anemic swine than in normal swine of the same weight.

The mean PCV at birth can be expected to be 35 to 37 per cent; by the tenth day of life the mean PCV value approaches 24–26 per cent, and this is followed by a rapid increase to reach and exceed the birth level during the next 2 weeks. A further increase in PCV may be expected as the pig matures giving a normal adult range of 30–47 per cent with a mean of 40–42 per cent.

During the last half of gestation the erythrocyte number decreases (Table 33) and continues to fall for some weeks after parturition. Among 20 sows in the third to eighth weeks of pregnancy the mean erythrocyte count was 6.9 million per cmm., among 14 sows 2 weeks before parturition the mean count was 5.7 million, and among 10 sows, the mean erythrocyte count was 4.9 million at 15 to 49 days post-partum. The PCV values were 43, 40 and 32, respectively, during gestation and 2–7 weeks post-partum.

Hemoglobin. The fluctuations observed in erythrocyte count and PCV are repeated in the concentration of hemoglobin during the growing period. Ranges may be anticipated as follows: birth = 8–13 grams per cent; 10th day = 4–9 grams per cent; 20 days = 9–11 grams per cent; 1–2 months = 11–12 grams per cent; and, in mature pigs = 10–15 grams per cent with a mean of 13 grams per cent.

Erythrocyte Life Span. Glycine-2-C¹⁴ was employed to study the life span of the red cell in 5 normal growing pigs.¹⁸¹ The mean survival time was estimated at 62 days, while the corrected average potential life span was 86 ± 11.5 days.

Erythrocyte Fragility. The pig erythrocyte is highly susceptible to hemolysis by hypotonic saline. Lyons¹⁵ reported initial hemolysis in 0.70 per cent saline and complete hemolysis at 0.45 per cent. Hudson¹⁸⁷ found fragility to be greater in blood of pigs less than 72 hours old than in pigs 7 months old. Sixty-five per cent of 20 samples from suckling pigs showed initial hemolysis in 0.80 per cent saline, whereas only 15 per cent of bloods of 7-month-old pigs revealed initial hemolysis in that concentration of saline.

Sedimentation Rate of Erythrocytes. The pig erythrocytes exhibit a propensity for rouleau formation and, therefore, the sedimentation test should be applicable. A diphasic sedimentation phenomenon is observed in suckling pigs during the period of most intense erythrogenesis. The observations on mature swine blood need to be extended before conclusions can be drawn regarding the anticipated sedimentation rates for normal blood. It appears from the limited data reported in Table 33 that rather high erythrocyte sedimentation rates can be anticipated in normal swine.

Leukocyte Counts, Total and Differential. The total leukocyte counts, as determined in one litter of 9 pigs, ranged between 5,600 and 19,100 during the first 3 weeks of life with mean counts between 8 and 11 thousand. Nine other pigs, 2 months of age, selected at random from a larger group revealed total counts of 16,000 to 25,000 with one pig having a count of 44,800. Excluding this latter count, the mean was 21,500. Total counts on the blood of 32 sows, revealed a range of 11,300 to 22,250 with a mean of 15,900. These results are in close agreement with reports to be found in the literature.^{19,164,170,184,189,192,193}

The differential leukocyte counts are characterized by a very high percentage of neutrophils at birth (70 per cent). By the end of the first week of life the neutrophils and lymphocytes are present in about equal numbers (45 per cent) but the former continue to drop (35 per cent) and latter continue to rise (55 per cent).^{170,183,185} The differential distribution in the adult pigs as given by Scarborough¹⁹ is as follows:

	<i>Neutrophil</i>	<i>Lymphocyte</i>	<i>Monocyte</i>	<i>Eosinophil</i>	<i>Basophil</i>
Range . . .	30-50	40-60	1-10	1-10	0-4
Mean . . .	39.0	52.0	3.3	4.5	1.2

The total and differential leukocyte counts of the sow show the stress pattern at parturition.^{190,191} A left shift including myelocytes, metamyelocytes and band neutrophils was observed 10 to 24 hours post-partum, Table 33.

The following is an example of the leukocyte picture as determined in one sow at parturition.

Differential Leukocyte Count in Percentage

<i>Time</i>	<i>WBC</i>	<i>Differential Leukocyte Count in Percentage</i>						
		<i>Meta.</i>	<i>Band</i>	<i>Neut.</i>	<i>Lymph.</i>	<i>Mono.</i>	<i>Eos.</i>	<i>Bas.</i>
Just before parturition . .	11.8	0	2.5	55.0	34.0	5.0	2.0	0
2 hours Post-partum . .	15.1	0	0	45.5	39.5	4.5	11.5	0
12 hours P.P. . . .	8.9	1.0	25.0	27.5	39.0	6.5	1.0	0
24 hours P.P. . . .	8.55	4.0	6.0	41.5	33.5	6.0	1.0	0

The Myeloid-Erythroid Ratio. Lahey *et al.*¹⁸⁸ report the leukocyte-erythroid ratio in 10 normal swine to be 1.77 ± 0.52 .

Thrombocytes. The number was reported to be $520,000 \pm 195,000$ in 10 normal swine.¹⁸⁸

Icterus Index. Icterus index was less than 5 units. Plant pigments in the diet do not appear in the blood plasma of swine.^{15a}

Specific Gravity. King and Wilson^{187a} reported the range in 43 normal swine to be 1.055–1.061, with a mean of 1.059. Palmer¹⁹² gave the range as 1.027–1.071, mean 1.061.

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Chapter 5

Hematopoiesis

HEMATOPOIESIS or hemopoiesis means to make blood. The hemato-poietic system is widely distributed throughout the body and includes a number of organs having functions in addition to blood formation.

<i>Tissue or Organ</i>	<i>Function in Hematopoiesis</i>
Bone marrow	Produces erythrocytes, granulocytes, thrombocytes and hemoglobin; stores iron.
Lymph nodes and follicles	Produce lymphocytes and participate in antibody production.
Liver	Stores B ₁₂ , folic acid, and iron. Produces prothrombin and fibrinogen. Converts free bilirubin to bilirubin glucuronide for excretion via the bile. Retains its embryonic potential for hematopoiesis.
Spleen	Produces lymphocytes, stores erythrocytes and iron, retains embryonic potential for hematopoiesis. Destroys erythrocytes and hemoglobin through its extensive R.E. system.
Stomach	Produces HCl for release of iron from complex organic molecules. Produces intrinsic factor which functions in preparing B ₁₂ for absorption by the intestinal mucosa.
Reticuloendothelial system	Produces monocytes. Destroys erythrocytes and converts hemoglobin to iron, globin and free bilirubin. Stores iron.
Kidney	Presumptive organ for production of erythropoietin.

HEMATOPOIESIS IN THE EMBRYO AND EARLY POST-NATAL LIFE

The formation of blood in the bovine embryo and fetus has been described by Winqvist²² and his work forms the basis for the material presented here. Winqvist relates the beginning of hematopoiesis and its duration in each organ to crown-rump length of the embryo. In order to visualize the information in terms of days in gestation, Table 34 is provided from data gathered by Schalm.²¹

Hepatic Hematopoiesis. The smallest embryo examined by Winqvist was 0.4 cm. in length and all blood cell formation was noted to be

intravascular. Nearly all blood cells were nucleated primitive red blood corpuscles, many of which divided mitotically.

In embryos 0.4 to 0.8 cm., the liver showed considerable development and in this organ the first evidence of extravascular blood cell formation was noted. Blood stem cells formed by transition from mesenchymal cells situated immediately outside the liver sinusoids. Hepatic hematopoiesis was very active in the 1.0 cm. embryo. Erythropoiesis predominated with great numbers of prorubricytes, basophilic and polychromatophilic rubricytes, and metarubricytes. In addition, a large number of

Table 34. Crown-Rump Length of 17 Bovine Embryos and Fetuses Correlated with Days in Gestation

<i>Days in Gestation*</i>	<i>Length in Centimeters</i>
28	0.6
48	3.5
56	4.8
68	9.9
70	9.5
80	13.0
99	19.0
113	27.2
122	31.5
141	36.0
154	38.0 (twins)
154	41.0
161	42.0
184	48.5
238	77.0
266	85.0
284	97.5 (stillborn)

*All Holstein embryos except the 13.0 cm. embryo which was of the Jersey breed.

megakaryocytes at various stages of development were observed between the liver cells. A few neutrophilic myelocytes could be detected in 1.2 to 1.3 cm. embryos both in the circulating blood and the hematopoietic foci of the liver. Also, typical thrombocytes were present in the blood. Rubricytes and definitive erythrocytes were observed in the blood and especially in the vascular bed of the liver in the 1.8 cm. embryo. Hepatic hematopoiesis remained a prominent feature in fetuses up to 35 cm. in length after which the hematopoietic foci decreased in size and shortly before birth hepatic hematopoiesis was almost completely ended.

Hematopoiesis in the Spleen and Lymph Nodes. Hematopoiesis began in the spleen at about the 7.0 cm. stage and it was a prominent feature in this organ in fetuses up to 60 cm. in length, after which, it gradually subsided. Erythrocytes, granulocytes, lymphocytes and megakaryocytes were formed in the spleen as well as in other lymphocytic foci. Even in newborn calves, the prefemoral lymph nodes were observed to be a site of lively formation of neutrophils from primitive reticulum cells.

The bovine has haemolymph nodes and in a 39 cm. fetus some erythropoietic activity was noted in such nodes. In newborn calves, erythropoiesis was no longer detected but granulopoiesis was apparent and continued in post-natal life in animals at least up to 2 years of age. Phagocytosis of erythrocytes by fixed reticulum cells of the haemolymph nodes occurred in calves and became a characteristic feature in such tissues of adult cattle.

The Prenatal Bone Marrow. Bones originate from the mesoderm and the marrow cavity is formed by vascularization of the primitive cartilaginous structures.

In the 18-cm. bovine embryo the newly formed marrow cavity contained erythropoietic foci, megakaryocytes of varying maturity, and a small number of immature neutrophils and eosinophils. Erythropoiesis predominated for the Myeloid:Erythroid ratio was less than 0.1:1.0. It was observed that extrusion of nuclei from metarubricytes was common at this as well as later stages and appeared to be the means of erythrocyte denucleation.

All hematopoietic centers were situated extravascularly. Nucleated erythrocytes were abundant just outside the walls of the sinusoids but were very rare inside the vessels. At the 30-cm. stage, considerable numbers of neutrophilic myelocytes and metamyelocytes were present and in the 50-cm. fetus, the marrow had begun to contain an appreciable number of fat cells. Lymphocytic foci were not found in the bovine bone marrow, although nodule-like aggregations of lymphocytes have been described in human bone marrow.

Embryonic Hematopoiesis Related to the Post-natal State. In many mammals, leukocytes are scarce throughout fetal life. However, in the bovine, the white cell distribution in fetal blood approached that of early post-natal life by the 70-cm. stage. A conspicuous difference between the blood picture of large fetuses and that of newborn calves is the almost complete absence of eosinophils in the blood of the latter.

The M:E ratio in fetuses from 18 to 90 cm. averaged 0.22 ± 0.04 as compared with 0.47 ± 0.06 for calves between 3 and 36 days of age and 0.64 ± 0.13 for bovines between 2 months and 10 years of age.

The erythrocytes at birth are larger than the red cells produced during the post-natal growing period (Table 15 and 16, pp. 147 and 152) and they contain fetal hemoglobin which takes up oxygen more readily at low gas tension. These fetal erythrocytes are replaced shortly after birth by corpuscles better suited to serve the developing young.

THE HEMATOPOIETIC SYSTEM IN GROWING AND ADULT ANIMALS

After birth, in the normal animal, erythropoiesis and granulopoiesis are confined to the bone marrow. At first the marrow of all bones is

active in blood cell production. During the phase of rapid growth of the young, the blood volume is expanding and placing heavy demands on the marrow for erythrocytes, and in addition the population of fetal erythrocytes must be replaced. This strain on erythropoiesis is dramatically illustrated in the pig during the first few weeks of life (Table 32, p. 190). As the demand for new erythrocytes decreases with approaching maturity, some red marrow is replaced by yellow marrow. This occurs essentially in the shafts of the long bones leaving active hematopoiesis to the warm, flat bones such as the sternum, ribs, pelvis, vertebrae, and skull. Some red marrow persists in the epiphyses of the long bones.

The yellow marrow is limited to three types of cells, namely (*a*) reticulum cells which are connected with the endosteum and blood capillaries, (*b*) the endothelium forming the walls of capillaries and sinusoids, and (*c*) the fat cells. The fat cells act to occupy space as hematopoiesis recedes and to give up space as demand for expansion of red marrow occurs. Transition of yellow to red marrow may take place in 48 hours after massive loss or destruction of erythrocytes⁵. Yellow marrow contains DL-batyl alcohol which is effective against granulocytopenia but its mode of action is unknown.

There are two schools of thought relative to the sites of origin of the erythrocytes and granulocytes. One opinion is that the erythrocytes are developed intravascularly from the endothelium of collapsed capillaries and that the granulocytes are formed extravascularly from reticulum cells. The second opinion is that both the erythrocytes and granulocytes are formed extravascularly from a multipotential primitive stem cell of the reticulum. The intravascular theory of erythrocyte production proposes that erythropoiesis takes place in collapsed capillaries, filling and distending them, and by eventual growth pressure the mature cells are pushed into the circulation. The theory of extravascular erythropoiesis states that the mature erythrocytes are delivered to the blood stream through the thin walled capillaries in a manner as to cause no actual rupture. All agree that the granulocytes are produced extravascularly and move into the vascular bed under their own power or by growth pressure.

It would appear from the reports of Sabin^{6,20} and McDonald¹⁵ that intravascular erythropoiesis is to be found in the pigeon, although Jordan¹³ disagrees. Drinker⁷ reports the extravascular occurrence of both erythropoiesis and granulopoiesis in the dog, cat and rabbit.

Bone Marrow Sections. The pigeon is a useful experimental subject for study of the theory of site of origin of erythrocytes and the granulocytic leukocytes. The marrow of the radius and ulna can be simplified or rendered hypoplastic by subjecting the birds to starvation. After 10 days to 2 weeks, during which only water is allowed, the marrow of the radius and ulna becomes essentially devoid of hemopoietic activity.

Food is now permitted and birds are sacrificed at 24-hour intervals to remove marrow for fixation and sectioning. By the end of 24 hours considerable cellular activity is apparent and the separate foci for erythropoiesis and granulopoiesis can be distinguished. The nests of immature red cells which can be identified by occurrence of an occasional hemoglobin containing cell are sharply delineated as if contained within vessels (Fig. 20). The developing granulocytes are easily distinguished

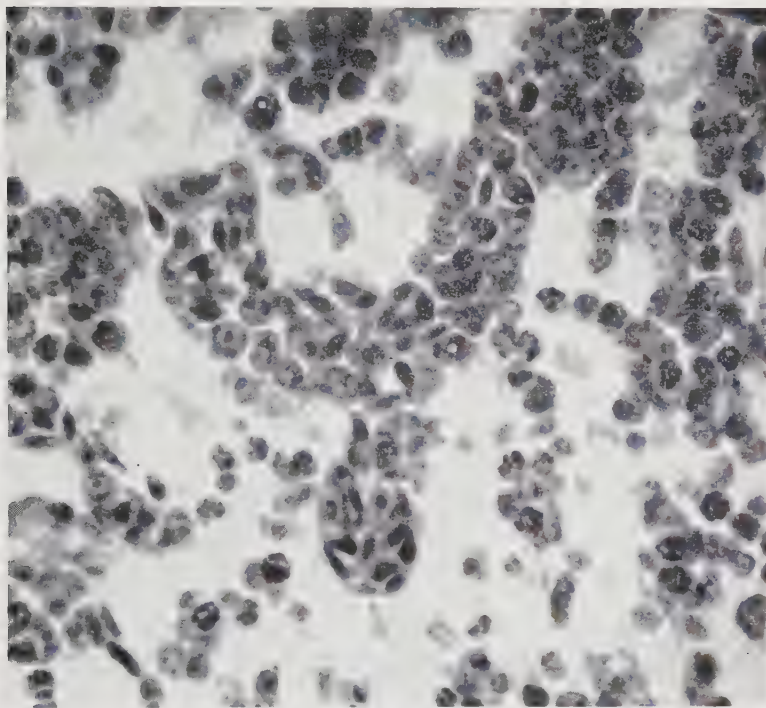


FIG. 20. Bone marrow section from the ulna of a young pigeon in which the marrow was first simplified by starvation. The Y-shaped cellular mass in the center is a site of active intravascular erythropoiesis.

by the eosin staining granules common to their cytoplasm in birds. The granulocytic cell nests are not sharply outlined and they appear between the fat cells and centers of erythropoiesis.

Rabbit bone marrow sections are useful for distinguishing the three cell types produced in mammalian bone marrow. The pseudo-eosinophil of rabbit blood is comparable to the neutrophil in other mammals. The eosinophilic granules make granulopoiesis readily distinguishable from erythropoiesis in rabbit marrow when eosin containing stains are employed. In addition, the thrombocytes of mammals are produced by

giant cells called megakaryocytes²³ and these cells are readily apparent in marrow sections. Birds do not have megakaryocytes, although they have nucleated cells in peripheral circulation which function similarly to the thrombocytes of mammals.

Bone Marrow Aspirations. Aspirated marrow (Chapter 2, p. 77) is more satisfactory for the study of cell morphology than tissue sections. It is best to aspirate in a dry syringe and prepare the film immediately. The nucleated cells in aspirated bone marrow are classified as belonging to either the erythrocytic or granulocytic maturation series as follows (Plates I, p. 97 and II, p. 103):

<i>Erythrocytic</i>	<i>Granulocytic</i>
Rubriblast	Myeloblast
Prorubricyte	Progranulocyte
Rubricyte	Myelocyte (neutrophilic, eosinophilic or basophilic)
Metarubricyte	Metamyelocyte "
Reticulocyte	Band "
Erythrocyte	Neutrophil, Eosinophil or Basophil

The occasionally encountered lymphocytes and monocytes are generally considered to be of peripheral blood origin and are classified separately.

Knowledge relative to the cellular composition of aspirated bone marrow is of value in the diagnosis of leukemia and multiple myeloma. Marrow studies are important when anemia of undetermined cause exists. The Myeloid:Erythroid ratio may indicate an above normal erythropoiesis but it may be ineffective insofar as increasing the circulating red cell mass is concerned (Appendix, Case 21, p. 356). In other cases, the bone marrow study may reveal a maturation arrest in the early stages of the erythrocytic series of cells.

Vitamin B₁₂ and folic acid are two essential vitamins for all cell reproduction. These vitamins function in the process of cell division and appear to be involved in nucleoprotein formation. When erythropoiesis is normal, the cellular pattern is referred to as *normoblastic*. In a deficiency of vitamins B₁₂ and/or folic acid there is maturation arrest in the prorubricyte and basophilic rubricyte stage. These large blue cells are then the prominent cells of the erythrocytic series in the marrow. Their nuclear chromatin is more coarse or particulate. The term *megaloblast* is used to designate them as abnormal cells and the marrow is termed *megaloblastic*. The nucleus of *megaloblasts* eventually becomes smaller and pyknotic, while the cytoplasm remains larger than normal and as a result the fewer than normal red cells that are produced are macrocytic. A deficiency of Vitamins B₁₂ and/or folic acid also leads to a reduction in number of granulocytes and thrombocytes in the marrow,

and giant forms of metamyelocytes may occur as well as hypersegmentation of the nucleus of the definitive neutrophil in peripheral blood.

The abnormal cellular composition of the marrow and the resulting anemia, leukopenia, and thrombocytopenia produced by a deficiency of Vitamin B₁₂ is known as pernicious anemia in man, and it is caused by a lack of intrinsic factor produced by the stomach mucosa. True pernicious anemia has not been described as occurring under natural conditions in animals, although a few instances of anemia associated with a megaloblastic bone marrow have been seen in the dog in the University of California Veterinary Clinic (Appendix, Case 16, p. 350).

In anemia due to iron deficiency, the erythrocytic cell series of the marrow is hyperplastic due to an over abundance of small metarubricytes. Due to the lack of iron for hemoglobin formation, the metarubricytes continue to divide producing smaller than normal cells and eventually when the nucleus is extruded the erythrocyte is smaller than normal (Appendix, Case 25, p. 364).

Anemias associated with hemorrhage or hemolytic crisis are readily detected as such from the appearance of a considerable number of immature cells of the red cell series in peripheral blood (Appendix, Cases 17 and 18, p. 353). Anemia resulting from erythrocytic hypoplasia secondary to nephritis or chronic infection generally can be suspected from the absence of reticulocytosis in the face of advanced anemia (Appendix, Case 10, p. 343). Thus, bone marrow studies are not essential in hematopoietic disturbances that can be understood from history, physical findings and peripheral blood morphology. The equine differs from the other domestic animals in this regard since the peripheral blood does not exhibit evidence of reticulocytosis or other signs of erythrocyte immaturity when erythropoiesis is intensified in response to red cell loss or destruction (Appendix, Case 13, p. 347). For this reason, evaluation of the cellular composition of bone marrow may be required more frequently in the horse than in the other animals.

Bone Marrow Volume. The bone marrow, while widely distributed, constitutes an organ equal to about two-thirds the size of the liver. Fairman and Whipple⁸ reported that in 2 adult canine female littermates the marrow volumes were 289 ml. and 319 ml. or 2.4 per cent and 1.9 per cent of the body weights, respectively. In these dogs the marrow weights were 61 and 67 per cent, respectively, of the liver weights. Nye¹⁶ reporting on marrow volume of two rabbits found it to be 2.0 to 2.59 per cent of the body weight, and Yoffey²¹ states that functional bone marrow makes up 2 per cent of the body weight in guinea pigs.

Bone Marrow Transplants. In the atomic age, the danger of ionizing radiation to living tissue is of prime concern to the biologist. Studies

in animals exposed to atomic blasts at Bikini were summarized by Bellar² somewhat as follows: "The typical clinical picture of the Bikini animals exposed to a lethal but not overwhelming dose of ionizing radiation was a drop in peripheral lymphocytes within a few hours followed by a fall in segmented cells (granulocytes) in a few days. In a week or 10 days the red cells decreased in number. About this time the clotting and prothrombin time increased (prolonged). Bloody, tarry diarrhea developed in 2 to 3 days. The animals became hyperirritable and belligerent at times. Death from clinical symptoms was often unexpected. Bone marrow studies indicate that hemopoietic cells were destroyed; first erythroblasts, then myelocytes, and last megakaryocytes. Macrophages, reticular cells, and fat cells were resistant to injury." Bellar states that the lethal doses of total body radiation that kill 50 per cent in 30 days are: guinea pig, 200R; dog, 325R; goat, 350R; man, 450R; mouse, 530R; and rabbit, 800R. The pathology of total body radiation in dogs has been described by Gleiser.¹⁰

It has been demonstrated with mice and rats that they can be saved following lethal ionizing radiation by injection of either splenic material or bone marrow.⁴ The bone marrow is repopulated by the injected hematopoietic cells. It was possible to delay death in mice and save some by use of heterologous rat bone marrow. It is conjectured that ionizing radiation temporarily neutralizes the immunologic defenses against heterologous cells, but later the defenses may become re-established and the destruction of the new population of hematopoietic tissue takes place and death ensues.

The Lymph Nodes and Follicles. These are scattered throughout the body along lymph channels and are the main source of lymphocytes. The lymph nodes are supported by a network of reticular cells and fibers which contribute to the widely scattered reticuloendothelial system. The lymphocytes migrate into sinuses of the nodes and are carried *via* the lymphatics to the thoracic duct from whence they are dumped into the blood stream. The lymph nodes may serve as extramedullary sites for granulopoiesis. Lymphocytic tissue is depressed by adrenocortical activity and on the other hand hypertrophy of lymphocytic tissue results when there is hypofunctioning of the adrenal cortex. Atrophy of lymphocytic tissue resulting in lymphopenia is seen in chronic interstitial nephritis in the canine.

The Liver. This organ functions in a number of ways in maintaining the integrity of the blood vascular system. It is the main depot for storage of vitamin B₁₂ and folic acid and serves in the storage of excess iron. Two very important plasma proteins serving in blood clotting, namely, prothrombin and fibrinogen, are produced by the liver.

The liver functions in removing from the body the useless pigment bilirubin which is an end product of hemoglobin degradation. The

free bilirubin is derived from the catabolism of hemoglobin within the reticuloendothelial cells, especially in the spleen. The free bilirubin is brought by the blood plasma to the liver where it is conjugated with glucuronic acid and excreted in the bile. Free bilirubin does not pass into the urine but the conjugated form is capable of passing through the kidney. The presence of bile pigment in the urine reflects either occlusion of biliary canaliculi, as often occurs in hepatitis, or extrahepatic bile duct closure.

The Spleen. During post-natal life the spleen normally produces lymphocytes and monocytes. It may under conditions of stress on the bone marrow become the site of production of erythrocytes, granulocytes, and megakaryocytes.

Table 35. Effect of Strenuous Exercise in the Horse on Red Cell Mass and Total Leukocyte Count

<i>Date</i>	<i>Condition</i>	<i>RBC</i>	<i>PCV</i>	<i>Hb.</i>	<i>WBC</i>
Jan. 13	rest	7.97	40	13.3	8,050
	exercise	11.28	52	17.5	10,700
Jan. 26	rest	7.90	36.5	13.5	8,500
	exercise	10.80	50	17.0	10,200
Feb. 10	rest	10.60	53	17.0	9,250
	exercise	9.43	51.5	16.9	8,650
Feb. 25	rest	9.42	47	15.8	8,800
	exercise	11.02	54	17.5	9,550
Mar. 10	rest	8.32	43	14.5	9,000
	exercise	11.40	56	18.6	11,450

The spleen serves as a reservoir for erythrocytes, releasing them into the circulation on demand for increased oxygenation of the body as a whole. A spectacular example of the influence of strenuous exercise on the sudden increase in circulating red cell mass can be seen in the horse. An example is shown in Table 35 of a 6-year-old gelding that was bled when at rest followed by the drawing of a second blood sample about 15 minutes later and after the animal was made to run rapidly for one mile. The results of dual bleedings on 5 separate dates are included. Note that on February 10 the red cell mass was less after exercise rather than greater. It is presumed that something unknown had caused the animal to become excited while at rest and contraction of the spleen occurred before the animal was raced.

The spleen functions in defense. It is well known that calves are resistant to anaplasmosis but after removal of the spleen, they can be infected with *Anaplasma marginale*. Also, removal of the spleen may be followed by an acute flare up of haemobartonellosis in animals; the

parasite having been present in such animals as a latent infection as long as the spleen produced its inhibitory influence.

The spleen serves as the graveyard for worn-out and/or abnormal erythrocytes. Thus, in all hemolytic diseases, the spleen becomes markedly enlarged as a result of the concentration of abnormal erythrocytes in the organ. The spleen in health removes from circulation the erythrocytes as they age and in certain diseases state, the spleen may become overactive in red cell destruction and thus become an etiologic factor in the production of anemia (Appendix, Case 26, p. 365).

There is indirect evidence that the spleen exerts an inhibitory influence over erythropoiesis in the bone marrow. Removal of the spleen leads to certain changes in the erythrocyte peripheral blood picture. In the dog,¹⁴ following splenectomy, leptocytes or target cells appear in a few days and may be found for months thereafter. Thrombocytes, reticulocytes, and nucleated erythrocytes appear in above normal numbers and may persist for several months following splenectomy. These findings suggest that the spleen exercises some control over the bone marrow.

The Stomach. In man, the stomach plays an important role in preparing vitamin B₁₂ for absorption by the intestinal mucosa. Castle and Townsend³ discovered the "intrinsic" factor which is produced by the gastric mucosa. When this factor is absent in man, the condition called pernicious anemia develops. The intrinsic factor appears to be a mucoprotein and it is readily obtainable for therapeutic purposes from the gastric mucosa of the pig.¹⁸ However, pernicious anemia has not been reported to occur in animals and the removal of the stomach, duodenum, and first 30 centimeters of the jejunum in dogs¹ did not lead to the development of pernicious anemia. This suggests that either intrinsic factor is not limited in production to the stomach in the dog or that it is not required for the absorption of vitamin B₁₂ in the canine.

Another function of the stomach in hematopoiesis is the production of hydrochloric acid. Food iron is ionized by HCl to ferric iron which passes into the duodenum where it is reduced to ferrous iron in which form it can be taken up by the intestinal mucosa. Iron absorption is influenced by the availability of reducing agents in the gut, such as ascorbic acid, or it becomes unavailable for absorption if a high level of phosphates are present in the gut. Lack of HCl is called achlorhydria and the abnormality leads to development of anemia in man. Achlorhydria is not known to be of common occurrence in animals.

The intestinal mucosa appears to be selective in the absorption of iron in its own right.^{9,11} The iron taken into mucosal cells is united with a protein called apoferritin to form ferritin. When the mucosal cells become saturated with ferritin no more iron is absorbed; this is referred to as the mucosal block hypothesis. Ferritin in the mucosal cells is in equilibrium with iron stores of the body, and when iron in

storage falls below a critical level more iron is permitted to pass the intestinal mucosa. Iron in the form of ferritin is readily available for use by the bone marrow in the construction of the hemoglobin molecule. Excess iron is stored as ferritin in the liver, spleen and bone marrow. When hemoglobin is broken down in the removal of over-aged or abnormal erythrocytes, the iron becomes immediately available for conversion into hemoglobin again.

Iron, therefore, is essentially in a closed cycle for very little is lost from the body except by hemorrhage. An excess of iron in the tissues produces hemochromatosis. Such excess iron is irritating and leads to development of cirrhosis in the liver, spleen and pancreas. Repeated blood transfusions may overburden the body with iron and lead to hemochromatosis.

Ferritin is an invisible form of iron. Hemosiderin is similar to ferritin and the iron can be used for hemoglobin manufacture. Hemosiderin is visible as golden brown granules in macrophages of the reticuloendothelial cells. When hemorrhage occurs into the tissues or hemoglobin is released in massive amounts, the iron may appear in the RE cells of the immediate area as hemosiderin.

The Reticuloendothelial System. This system is widely scattered and consists of the macrophages of the loose connective tissue, the reticular cells of the spleen, the lymph nodes, and the bone marrow, the Kupffer cells of the liver and similar cells in the adrenal glands, hypophysis and lungs.

The RE cells are both fixed tissue cells and freely moving cells. They are considered by many to be the source of the blood monocytes; although, admittedly, this is debatable. The RE cells engulf and destroy cellular debris and particulate foreign material entering the body. They play a significant role in the catabolism of hemoglobin, especially in the spleen. The RE cells are capable of engulfing whole erythrocytes but more generally the over-aged erythrocytes go through a process of fragmentation¹⁹ to hemoglobin dust which is engulfed by the RE cells. The hemoglobin is broken down into iron and globin which are conserved and free bilirubin which is carried to the liver by the blood plasma where it is conjugated with glucuronic acid and excreted into the bile as bile pigment.

The Kidneys. Erythropoietin is now well established as a plasma factor elaborated in increasing amounts in response to anoxia and capable of stimulating erythropoiesis. A search for the organ or tissue of origin has drawn attention to the kidney as a probable organ of elaboration.¹² Rats subjected to removal of the pituitary, thyroid, spleen, adrenals or gonads retained the capacity to produce erythropoietin as well as rats from which seven-eighths of the liver had been removed. After bilateral nephrectomy, neither rats nor rabbits had the capacity to produce erythro-

poietin under the experimental conditions. Ureter-ligated rats, whose blood urea nitrogen concentration equalled that of nephrectomized animals, responded nearly like normal controls to bleeding or phenylhydrazine anemia, and erythropoietin was demonstrated in their plasma. The investigators were of the opinion that these findings demonstrate that urea retention in nephrectomized animals is not a depressing factor for erythropoiesis in uremia.¹⁷

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Chapter 6

The Erythrocytes: Their Production, Function and Destruction

THE mass of circulating red cells plus the erythropoietic tissue of the bone marrow constitute the erythron. Removal of blood or bone marrow from the living animal is comparable to the examination of other organs or tissues by biopsy. Pathologic and physiologic states may affect the erythron in a manner to result in (*a*) atrophy or anemia, (*b*) hypertrophy or polycythemia, (*c*) dehydration or hemoconcentration, and (*d*) hydremia or hemodilution.

A number of hormones play a physiologic role in control of the size of the erythron.⁵ This is shown by the following: In both man and the rat it is reported that removal of the gonads results in a change in the number of circulating erythrocytes. The count increases in females following removal of the ovaries and falls in males after castration. Parenteral administration of the respective hormone to the castrated individuals causes the erythrocyte count to return to the normal level for the sex in question. Anemia is associated with hypothyroidism (Appendix, Case 12, p. 345), adrenal cortical hypofunction (Addison's disease), anterior pituitary insufficiency (Simmond's disease) and splenomegaly (Banti's disease). On the other hand, polycythemia is observed in hyperfunction of the adrenal cortex (Cushing's disease). The erythron is kept in delicate balance as shown by the fact that, in health, the daily production of erythrocytes equals the daily loss from destruction of over-aged cells. The rate at which the erythrocytes are destroyed and replaced can be visualized by the following hypothetical case: A 30 pound dog has 1,200 ml. of blood on a basis of 40 ml. per pound of body weight. If the red cell count is 7,000,000 per cmm., then the number per ml. of blood will be 7,000,000,000. The average life span of the canine erythrocyte is 120 days and thus the erythrocyte turnover rate would be $7,000,000,000 \times 1,200 \div 120$ or 70 billion erythrocytes replaced daily or about 800,000 every second.

THE FUNDAMENTAL STIMULUS FOR ERYTHROPOIESIS

It has been known for years that anoxia stimulates the normal bone marrow to increase its production and release of erythrocytes. It is

also a well known fact that the administration of cobalt leads to intensification of erythropoiesis. As recently as 1952, Grant and Root,⁶ in a review of the fundamental stimulus for erythropoiesis were unable to explain the true nature of the stimulus. Reference was made to a concept put forward in 1906 by Paul Carnot in which he proposed the production of a humoral agent, hemopoietine, by some organ or tissue which in turn stimulated erythropoiesis. The trigger mechanism being either anoxic anoxia or anemic anoxia. Reissmann⁹ produced chronic hypoxemia in one partner of a pair of parabiotic rats and observed erythropoiesis to be stimulated in the bone marrow of both rats of the pair. It was concluded that a humoral factor produced in the rat under reduced oxygen tension passed into the circulation of its parabiotic partner and stimulated its bone marrow. Erslev¹ showed that normal rabbit plasma injected into normal rabbits did not stimulate red cell production but plasma from rabbits first made anemic had a stimulating effect on erythropoiesis when injected into normal rabbits. The repeated injection of anemic rabbit plasma into normal rabbits produced a significant rise of reticulocytes, erythrocytes, and hemoglobin.³ Recent studies point to the kidneys as one of the probable sources of erythropoietin and it has been suggested that cobalt stimulates erythropoiesis by producing a local anoxic state in the kidney.^{4,8} Erythropoietin is not species specific for anemic rabbit plasma is effective in mice and anemic dog plasma stimulates erythropoiesis in rats.⁷

Erslev² investigated the level of cell mitosis at which the anoxic stimulus acts. Since prolonged anoxia can reactivate dormant yellow marrow, it appears reasonable that the stimulus acts at the level of the undifferentiated stem cell. By a series of experiments in rabbits, Erslev concluded that "the anoxic stimulus operates in the bone marrow by accelerating the differentiation of stem cells into pronormoblasts (pro-rubricyte) and that thereafter the maturation and multiplication of differentiated nucleated red cells proceed at fixed rates independent of the anoxic stimulus."

The true nucleoli and the cytoplasm of the rubriblast, prorubricyte and basophilic rubricyte take the basic stain (blue) due to their content of ribonucleic acid (RNA). The nucleus is more purplish and contains deoxyribose nucleic acid (DNA). Immediately before mitosis takes place the DNA content of the nucleus doubles.¹⁰ The nucleolus disappears and its substance diffuses toward the nuclear membrane, on the outside of which an intense production of RNA takes place. It is conjectured that this RNA moves across the nuclear membrane and provides the substance for doubling of the DNA which is required for chromosome production. Vitamin B₁₂ and folic acid are essential for DNA elaboration in the nucleus just before visible mitosis. When there is

a deficiency of these essential vitamins, a maturation arrest occurs resulting in abnormal basophilic cells in the marrow called megaloblasts.

The first phase of erythropoiesis is concerned with the synthesis of primitive cellular protein which leads to an increase in the total number of cells, prorubricyte-basophilic rubricyte stages. The nucleolus loses its RNA in the initial cell division and thereafter when present it appears as a ring-like structure. The next phase leads to a decline in cytoplasmic RNA. The cytoplasm loses its basophilic staining, and the first sign of hemoglobin synthesis (polychromatophilia) makes its appearance. The nucleus loses its potential for mitotic division, becomes pyknotic, and disappears. The cell now has reached the stage immediately prior to that of the mature erythrocyte which is called the reticulocyte (Plate II, p. 103).

THE RETICULOCYTE

The time required for release of the new red cells in large numbers to peripheral blood following maximum anoxic stimulation is about 4 days. In acute experimental anaplasmosis (Appendix, Case 17, p. 353), the polychromatophilic erythrocytes or reticulocytes appear in peripheral blood on about the fourth day after massive removal of the parasitized erythrocytes, with the peak of activity being reached by the seventh day.

The size of the cell, in the erythrocyte maturation series, decreases as the cell matures. Thus, the reticulocyte, which is still immature is larger than the definitive erythrocyte. When reticulocytes are released in large numbers, the mean corpuscular volume (MCV) increases in proportion to the number of immature cells present in the circulation. Referring to Appendix, Case 17, p. 353, it is seen that the normal MCV in this cow was 40-50 cubic microns and in remission following hemolytic crisis, the MCV reached 117 μ , or almost $3\times$ normal red cell size. These large polychromatophilic cells disappear in a few days from the circulation without a fall occurring in the erythrocyte count. This suggests that hemoglobin synthesis¹⁴ is completed in the circulation and that the mean red cell size diminishes through loss of water and concentration of the hemoglobin.

Brilliant cresyl blue in 1 per cent solution in physiologic saline is used to demonstrate reticulocytes (Chapter 2, p. 48). This vital stain produces a bluish stippling in the central area of the reticulocyte (Plate II, p. 103). The amount of this material varies from cell to cell, depending on the stage of ripening or maturation. The reticulum is believed to be composed of RNA which disappears as the reticulocyte matures into an erythrocyte.¹⁶ The ripening time of the reticulocyte in peripheral blood in the dog was found to vary between 19 and 43 hours with an average of 31 hours.¹⁵

Reticulocytes are not found in health in the blood of the horse, cow, sheep and goat. This means that the reticulocytes ripen in the bone marrow of these animals. The dog and cat may normally have an average of 0.5 to 1.0 per cent reticulated cells in peripheral blood and swine may have up to 2 per cent. The rabbit, guinea pig, rat, and mouse are good subjects from which to obtain blood for the demonstration of reticulocytes for the blood of these animals normally contains between 2 and 4 per cent reticulocytes.

Reticulocytes increase in peripheral blood during response of the bone marrow to blood loss, hemolytic diseases, or in remission of other types of anemia (Appendix, Cases 14, 15, 16, 17, and 18). However, limited experience with the equine suggests that reticulocytosis may not be a prominent feature in this species during active blood regeneration (Appendix, Case 13, p. 347).

The reticulocyte differs from the mature red cell in that it is a larger cell (macrocyte); it is of lower specific gravity and it is more resistant to crenation¹³ (Fig. 9-1, p. 52); it does not participate in formation of normal rouleau or pathologic agglutination;¹⁷ it is more resistant to specific hemolytic serum¹¹; it may be more resistant to hypotonic saline¹² although results with this characteristic are debatable^{13,18}; and the reticulocyte is less susceptible to mechanical trauma *in vitro*.¹⁸ All of these characteristics are of a protective nature to provide the cell with a greater potential for survival in the circulation than is shown by the mature erythrocyte. This naturally greater resistance to forces that may destroy the mature cell suggests a mechanism whereby hemoglobin can be contributed to the circulation under conditions favoring destruction of mature erythrocytes. In anaplasmosis, the *Anaplasma marginale* body does not attack reticulocytes as these cells pour into the circulation to replace the destroyed parasitized mature erythrocytes (Plate IX-4, p. 247).

ESSENTIAL MATERIALS FOR ERYTHROCYTE PRODUCTION

Protein. A dog that is maintained in an anemic state by repeated blood removal and fed a low protein diet is unable to produce the usual amount of globin for hemoglobin synthesis even in the presence of a large excess of iron.²⁰ The anemic dog kept on a protein fasting diet was found to utilize protein from its own tissues to produce hemoglobin and plasma protein.²³ For every kilogram of weight loss, 50-140 grams of blood protein were produced. Thus, the body attempts to correct the anemia and hypoproteinemia by raiding the body tissues. Casein, lactalbumin, whole egg protein or liver protein in amounts of 150-250 grams of protein/week were able to maintain body weight, a strong positive nitrogen balance, and produce considerable amounts of new hemoglobin

and plasma proteins.²¹ In the rat it was shown that protein intake is more essential for maintenance of normal erythropoiesis than is total caloric intake.¹⁹ Removal of protein from the diet was followed promptly by hemoconcentration, diminution of blood volume, and drastic reduction in erythropoiesis. These changes were reversible upon addition of protein to the diet.

Whipple and associates²⁰⁻²² have made extensive studies on the value of various common diet factors for supporting hemoglobin production in dogs made anemic by bleeding. Some results of their trials are summarized in Table 36. Dogs were observed to store ingredients for hemo-

Table 36. Regeneration of Hemoglobin in Dogs Made Anemic by Bleeding and Fed Various Common Diet Factors

<i>Diet factor in grams administered daily</i>	<i>Grams of hemoglobin produced over a 2-week feeding period</i>
Bread, 400	3
Milk 450; bread, 400	3
Cream, 100; bread, 400	10
Butter, 100; bread, 350	15
Asparagus, 200; bread, 300	9
Spinach, 200; bread, 300	15
Raisins, 200; bread, 300	25
Apricots, 200; bread, 300	48
Eggs, 150; bread, 300	45
Whole fish, 250; bread, 300	13
Beef muscle, 250; bread, 300	17
Pig muscle, 250; bread, 300	30
Chicken gizzard, 250; bread, 200	80
Kidney, 250; bread, 300	70
Chicken liver, 250; bread, 300	80
Beef liver, 300; bread, 300	80
Beef liver, 450	95

globin formation during periods of favorable diet. These carry-over materials rarely lasted more than two weeks under conditions of anemia from bleeding and an unfavorable diet. A dog in health on an adequate diet readily replaces hemoglobin lost by hemorrhage.

Minerals. Iron, copper, and cobalt are the principle minerals required for red cell production. *Iron* is an integral part of the hemoglobin molecule and therefore it is absolutely essential for hemoglobin synthesis. *Copper* is required in trace amounts and appears to serve as a catalyst for mobilization of iron for hemoglobin synthesis. *Cobalt* is essential to the diet of ruminants. Vitamin B₁₂ is synthesized in the rumen and cobalt is required in the B₁₂ molecule. Cobalt administered to animals in excess causes an increase in the red cell number and leads to polycythemia.

Vitamins. The essential vitamins for erythropoiesis are in the B

series and consist of riboflavin (B_2), pyridoxine (B_6), niacin or nicotinic acid, folic acid, thiamin, and B_{12} . All may not be required by every species of animal but deficiencies may lead to development of anemia in one or more animal types.

STRUCTURE AND FUNCTION OF THE ERYTHROCYTE

The red cell is composed of water, 60–70 per cent; hemoglobin, 28–35 per cent (MCHC); and a matrix of organic and inorganic materials which contribute up to 5 per cent of the red cell volume. The cell membrane is flexible but essentially nonelastic. Thus, there is a critical volume beyond which the cell membrane ruptures. Hemolysis takes place when an erythrocyte becomes spherical as a result of taking on water beyond the critical volume. The different mammal erythrocytes show different fragilities in hypotonic solutions and when arranged in order of mean erythrocyte volume (MCV) from the largest to the smallest cell, the arrangement is also essentially in order of increasing susceptibility to hypotonic saline solution.²⁸ The pig red cell which is very susceptible to hemolysis does not follow this general pattern. The equine erythrocyte is more resistant, according to the few reports in the literature, than its general position in the scheme would suggest it should be.

Castle and Daland²⁶ have suggested that the differences in susceptibilities of red cells of various animal species to hemolysis in hypotonic saline may be related to differences in form rather than to osmotic behavior. The biconcave shape should permit more swelling in hypotonic solution without significant increase in original surface area whereas cells with less biconcavity would reach their critical volume much more quickly and therefore would hemolyze more readily.

The mammalian erythrocyte is anuclear, while all other vertebrates have nucleated red cells. Among the domestic animals the canine erythrocyte is distinctly biconcave and this is readily apparent by the distinct central pallor in stained films (Plate III, p. 109). The cat and horse erythrocytes generally stain diffusely or with only limited evidence of central pallor (Plates IV and VII, pp. 113 and 123) while the red cells of the cow, sheep, goat, and pig may or may not present a pale central area (Plates V, p. 117, VI, p. 119 and VIII, p. 127). Thus, except for the dog, the red cells of the common domestic animals are not distinctly biconcave in shape but tend more toward the flat disk with little or no central depression. The family Camellidae, which includes the camel, alpaca, and llama, is distinctive in having elliptical erythrocytes and some members of the deer family may have sickled red cells.

The primary function of the erythrocyte is to serve as a carrier of hemoglobin. The incorporation of hemoglobin in a special cell for circulation in the blood stream makes possible the complex structure

and specialized activities of the vertebrates. Hemoglobin in solution in the concentration found in mammals would greatly increase the viscosity of the blood and it would exert an osmotic pressure about 5 times greater than normal plasma. To circulate such a blood and bring about an exchange of water between blood and tissues would require a tremendous increase in work-load of the heart, as well as significant changes in the structure of the blood vessels and the capillary bed. All this is avoided by placing hemoglobin within a cell that is capable of passing through the bore of the smallest capillary. Another advantage is gained, for in the interior of the erythrocyte, the hemoglobin exists in an environment which is designed to take advantage of the pH, slightly more acid than plasma, at which hemoglobin is most efficient as a respiratory pigment.^{24,25} Another function of the erythrocyte is to contribute to blood volume and thereby participate in the dynamics of blood circulation. The volume per cent of erythrocytes (PCV) in blood varies with animal species but mean values in the common domestic mammals fall between 35 and 45 per cent.

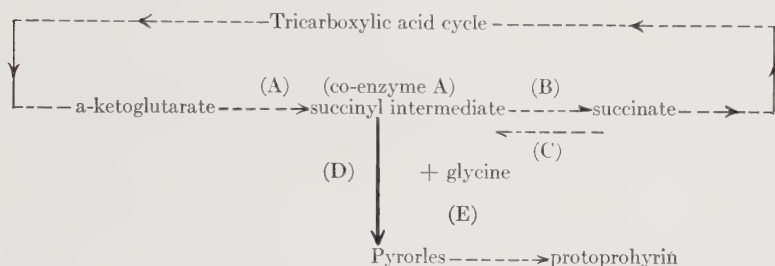
The stromal framework of the erythrocyte supports the hemoglobin and the two are bonded together. When an erythrocyte undergoes fragmentation, the hemoglobin does not escape but remains with the fragments. Hemoglobin is released in free form in the condition called hemolysis in which case the bond between the hemoglobin and stroma is broken by the hemolytic agent. Free hemoglobin in blood plasma is quickly disposed of by being oxidized to useless forms, lost through the kidneys and/or destroyed by the reticuloendothelial system.²⁷

HEMOGLOBIN SYNTHESIS AND DESTRUCTION

Hemoglobin Synthesis. Hemoglobin consists of protoporphyrin, iron, and globin. The basic structure consists of 4 pyrrol rings linked together by 4 methene bridges to form the porphin nucleus common to all porphyrins.^{29,39} The porphin nucleus has 8 possible sites for substitution of side chains. By replacing the 8 hydrogen atoms with 4 methyl ($-\text{CH}_3$) and 4 ethyl ($-\text{CH}_2-\text{CH}_3$) groups, 4 isomeric compounds can be derived. These have been designated etioporphyrins and are called types I, II, III, IV. Only types I and III porphyrins are found naturally; the protoporphyrin of hemoglobin is classified as protoporphyrin 9, type III.

Heme is protoporphyrin plus ferrous iron. This chemical structure is synthesized in the red cell in the formation of hemoglobin. Glycine labeled with N^{15} was administered to ducks and humans⁴¹ and the heme was isolated from the red cells. It was demonstrated that glycine is a precursor for nitrogen in both types of pyrroles in the protoporphyrin molecule. Through the use of glycine labeled with C^{14} in the α -carbon

atom, it was concluded that the 4 carbon atoms of the methene bridges come from this source and that 4 additional carbon atoms are supplied to the pyrrol structure.⁴² The remaining 26 carbon atoms in the porphyrin molecule were shown to be derived from acetate which is utilized by being converted to a 4-carbon atom unsymmetrical compound *via* the tricarboxylic acid cycle.^{37,38}



Under normal conditions, hemoglobin is synthesized before the erythrocyte nucleus is lost but during intense erythropoiesis many immature erythrocytes, that is polychromatophilic erythrocytes, are released to peripheral blood (Plate IX-1, p. 247). Hemoglobin synthesis in polychromatophilic red cells is incomplete and the question arises as to whether or not these cells are able to complete the synthesis of hemoglobin in the blood stream. Blood from rabbits made anemic by bleeding or phenylhydrazine hemolysis was incubated *in vitro* with glycine labeled with N^{15} and after 24 hours heme was prepared from the red cells and the N^{15} concentration determined.¹⁴ The authors concluded that the non-nucleated but immature mammalian erythrocyte is capable of synthesizing heme *in vitro* while significant synthesis does not occur with normal mature erythrocytes. Thus, when immature erythrocytes enter the circulation hemoglobin synthesis may continue bringing the cell to maturity.

In addition to protoporphyrin of the hemoglobin molecule, two other natural porphyrins, coproporphyrin and uroporphyrin, are found in trace amounts in feces and urine and the former also occurs naturally in young erythrocytes.³⁵ Coproporphyrin excretion is increased whenever erythropoiesis is intensified. Porphyrinuria is a term which is applied to increased excretion of porphyrins in the urine. An example, in addition to the increase secretion following hemorrhage and hemolytic diseases, is the occurrence of coproporphyrin III in abnormal amounts in the urine in lead poisoning. Porphyria is a disease of porphyrin metabolism and is generally of congenital origin. Both coproporphyrin and uroporphyrin are produced in excessive amounts and the latter has a predilection for the teeth and bones causing these structures to take on

a brown or reddish color. Congenital porphyria of cattle is called "Pink Tooth" because of brown to reddish color of the teeth³⁴ (Appendix, Case 23, p. 361). The urine is brown to wine color as a result of the large amount of porphyrins present. The porphyrins have photosensitizing characteristics and the white areas of the body of cattle affected with congenital porphyria may show lesions typical of photosensitization.

Hemoglobin Destruction. Free hemoglobin may appear in the plasma in sufficient concentration to impart a pink or red color when massive destruction of red cells occurs in the vascular bed. The condition is called hemoglobinemia and when free hemoglobin is present in such amounts it passes the kidney threshold³² and appears in the urine. The latter condition is called hemoglobinuria (Appendix, Case 14, p. 347).

The more normal manner for hemoglobin to be destroyed is by splitting-off and reuse of the iron and globin and the discarding of the protoporphyrin molecule. The latter is converted to biliverdin (green pigment) in the RE system and this in turn is reduced to free bilirubin (yellow pigment) and carried by the blood plasma to the liver for excretion as bile pigment. The actual concentration of bilirubin in the plasma of the dog, cat, cow, sheep, and pig is very low, whereas a relatively large amount occurs in the plasma of the horse.⁴⁰ Birds excrete biliverdin into the bile, while the domestic mammals excrete bilirubin. However, in sheep, cattle, and pigs feeding on green vegetation, the bilirubin in the gallbladder may be partly oxidized to the green pigment, biliverdin.

The origin of bile pigments from hemoglobin was suspected by Virchow.³³ In 1847, he published the observation that a pigment resembling bilirubin could be found in tissue areas where previous hemorrhage had occurred. Virchow postulated that the pigment was derived from the hemoglobin of disintegrating erythrocytes. In 1889, Lowit³³ proclaimed that the phagocytic cells of the liver, spleen, bone marrow, and peripheral blood convert hemoglobin to bile pigments. The hypothesis is generally accepted that the catabolism of hemoglobin is a function of the reticuloendothelial system. By the injection of N¹⁵ labeled hematin (heme) intravenously into the dog, London³¹ demonstrated that hematin can be readily converted into bile pigment. One gram of hemoglobin gives rise to 35 mg. of bilirubin in the dog.³⁰ However, while hemoglobin is now regarded to be the main source of the bile pigments, it has been shown that other porphyrins, such as cytochrome pigments, *etc.*, also contribute in small measure to the total bile pigment source.

In 1913, van den Bergh and Snapper³³ introduced a test for bilirubin involving the use of diazotized sulfanilic acid to form the reddish pigment, azobilirubin. This technic has become widely known as the van den

Bergh test for bilirubin. By 1916, van den Bergh recognized that bilirubin occurs in two forms; the methods to detect these are called the "direct" and "indirect" van den Bergh tests for bilirubin. Free bilirubin as released to the blood plasma from the RE cells is insoluble in water but soluble in alcohol (indirect-reacting bilirubin). The free bilirubin is conjugated with glucuronic acid in the parenchymatous cells of the liver.³⁶ The bilirubin conjugate is soluble in water and reacts with the diazo reagent (direct reaction) without the addition of alcohol.

Free or prehepatic bilirubin does not enter the urine probably because of its insolubility in water, but posthepatic bilirubin (conjugated form) which is water soluble is able to pass the kidney into the urine. In icterus or jaundice, the van den Bergh test is often employed to aid in establishing the nature of the hyperbilirubinemia. In hemolytic diseases most of the bilirubin is prehepatic or indirect-reacting whereas in intra-hepatic obstruction or extrahepatic occlusion of the bile duct, the interference with free flow of bile into the intestine leads to regurgitation of direct-reacting bilirubin into the blood stream. Regurgitation of this form of bilirubin leads to the occurrence of bilirubin in the urine.

Generally, icterus due to posthepatic obstruction is not as intense as hemolytic icterus since the former pigment can escape into the urine. In severe hemolytic icterus both forms of bilirubin are usually found in the plasma. The anemia results in a partial anoxia which may lead to swelling of liver cells and partial closing of the bile canaliculi. A portion of the conjugated bilirubin is regurgitated into the blood and spills over into the urine.

A condition of hypobilirubinemia is commonly seen in chronic diseases such as infections, malignancies, and in advanced chronic interstitial nephritis in the dog (Appendix, Case 10, p. 343). This is due to the fact that such chronic diseases lead to depression of erythropoiesis and secondary anemia. As the number of erythrocytes available for removal by the RE system becomes reduced, the concentration of bilirubin in the plasma becomes less and less and the plasma in turn becomes devoid of visible pigment.

ERYTHROCYTE LIFE SPAN

Knowledge of the life span of the erythrocyte is of value in understanding the dynamics of red cell production and destruction. Life span studies in animals have indicated that the erythrocyte of each species has a characteristic mean survival time which is the result of both the potential life span and the loss of cells from random destruction irrespective of age.

Hawkins and Whipple⁶⁰ used bilirubin production as a measure of the length of life of the canine erythrocyte. A renal biliary fistula was

established and a significant reticulocytosis was produced by mass removal of blood. The peak of the reticulocyte curve was taken as the starting point. Bilirubin excretion decreased at first due to the large number of young red cells in the circulation. This was followed by a gradual increase in bilirubin excretion with a maximum output occurring between 112 and 133 days. This maximum production of bilirubin was interpreted to be due to mass death of the red cells which entered the circulation at the same time in response to the induced anemia.

Serologic technic for ascertaining the persistence of transfused erythrocytes in the circulation was developed by Ashby.⁴³ The recipient's blood is treated with specific immune serum to cause his red cells to agglutinate, leaving the donor or transfused cells unagglutinated for ease of detection. The post-transfusion survival of canine erythrocytes was 90–100 days when tested by differential agglutination employing canine anti-A serum.⁶⁵

Tagging or labeling erythrocytes with isotopes is considered to be a more accurate procedure. The isotopes commonly used for this purpose are Cr⁵¹, Fe⁵⁵, Fe⁵⁹, N¹⁵, C¹⁴. Each isotope has its own method of use and there are both advantages and disadvantages.⁵⁶ The Cr⁵¹ is a tagging method whereby the chromium becomes attached to the surface of the erythrocytes, while the other isotopes represent a labeling process; the specific isotope is incorporated in the hemoglobin molecule as it is synthesized in new cells. In the labeling process, the animal may first be made anemic by bleeding or by producing hemolysis of red cells by the injection of phenyldrazine. The isotope is then administered and incorporated in the new generation of erythrocytes produced in response to the induced anemia. Erythrocyte life span studies in various mammals are summarized in Table 37.

In hibernating animals the erythrocyte life is extended.⁴⁷ It was observed in the marmot (woodchuck) that during non-hibernation the mean life span of red cells was 36 ± 2 days but during hibernation this was extended to 112 ± 7 days.

Erythrocyte life span has been determined for some domestic birds. By the use of C¹⁴, the mean red cell survival time was found to be 20 days in the chicken and 39 days in the duck.⁴⁸ Using Cr⁵¹ the maximum erythrocyte life span was reported to be 35 days in the chicken, 35–45 days in the pigeon, and 42 days in the duck.⁶³

Erythrocyte survival studies were made in the Mule deer, Aoudad sheep, and Springbok antelope using glycine-2-C¹⁴. The survival time was expressed as half survival time or the point in time when one-half the labeled red cells had disappeared from the circulation. For the Mule deer and Springbok antelope the results were 95 and 80 days, respectively. It was of special interest that the Aoudad sheep used in

the study appeared to be the first apparently normal mammal to exhibit the presence of 2 distinctly separate populations of erythrocytes. The half-survival times of these two populations were 65 and 170 days, respectively.⁵⁴

An application of erythrocyte life span studies has been made in swine with certain induced anemias.^{51,52} In pyridoxine (B₆) deficiency, the erythrocyte survival time was normal but in folic acid deficiency and copper deficiency red cell survival time was decreased significantly.

Table 37. Erythrocyte Life Span in Various Animals Determined by Use of Isotopes

<i>Literature Reference</i>	<i>Species</i>	<i>Isotope Method</i>	<i>Mean Life Span in Days</i>
(64)	man	N ¹⁵	127
(18)	dog	Fe ⁵⁵ and Fe ⁵⁹	119-122
(59)	dog	Fe ⁵⁵	110
(57)	dog	Fe ⁵⁵	110
(49)	dog	Fe ⁵⁹	107
(45)	dog	C ¹⁴	115
(66)	cat	N ¹⁵	77
(49)	cat	Fe ⁵⁹	68
(53)	pig	C ¹⁴	62
(61)	pig	Fe ⁵⁹	63
(58)	pig	C ⁵¹	71
(44)	lambs 3 months old	Fe ⁵⁹	46
	lambs one year old	Fe ⁵⁹	52
	calves 3 months old	Fe ⁵⁹	55
(59)	rabbit	Fe ⁵⁵	50
(50)	rabbit	Fe ⁵⁵	45-50
(62)	rabbit	N ¹⁵	65-70
(49)	rabbit	Fe ⁵⁹	68
(50)	rat	Fe ⁵⁵	45-50
(59)	rat	Fe ⁵⁵	50
(46)	rat	C ¹⁴	68
(50)	mouse	Fe ⁵⁵	20-30
(59)	mouse	Fe ⁵⁵	25
(55)	horse	C ¹⁴	140-150

DESTRUCTION OF ERYTHROCYTES

The manner in which over-aged red cells are removed in the normal animal is a matter of conjecture. Rous and Robertson^{76,77} proposed the hypothesis of fragmentation without loss of hemoglobin; the fragmenting portions continuing to become smaller and smaller until hemoglobin dust is produced and removed by the reticuloendothelial cells. In support of this hypothesis, the authors state that poikilocytes (Plate IX-5, p. 247)

are not produced as such in the bone marrow but are found regularly in the spleen and only inconstantly in other organs. Doan and Sabin⁷⁰ confirmed the concept of fragmentation of red cells in the circulation of the normal rabbit by the study of films of fresh blood. They state as follows: "In watching fragmentation in sealed films of blood, we have found that a red cell puts out a long process and thereby becomes the so-called poikilocyte. At first the process does not move, then it begins to vibrate slowly, then faster and faster. This vibration marks the beginning of fragmentation. As the process vibrates, it gradually becomes thinner and thinner at some point, and then separates. If the thinned out place is at the base of the long process, the fragment becomes a rod; if near the tip, it becomes a round oval body. We have seen every variation in size and shape of these processes, up to an actual division of a red cell into two equal parts. Sometimes two or more processes form on a red cell at the same time. . . . We interpret the poikilocyte as the first stage in the process of fragmentation, and the microcyte, in many instances at least, as a red cell reduced in size after some of its cytoplasm has broken off."

When poikilocytes occur in peripheral blood in anemia, it is interpreted as a sign of early destruction of erythrocytes and indicates the existence of a vicious cycle of production and destruction of red cells.⁷⁷ The disease is of such a nature that the bone marrow is rendered unable to produce proper cells and those it does release are soon destroyed. A good example is Haemonchosis in the ovine (Appendix, Case 18, p. 353). A case of chronic lead poisoning in the dog revealed poikilocytosis (Appendix, Case 22, p. 360) and poikilocytes were frequent in a case of congenital prophyria in the bovine (Appendix, Case 23, p. 361). Poikilocytes may occur in chronic interstitial nephritis in the canine (Appendix, Cases 10 and 11, pp. 343 and 344).

Ponder⁷⁵ states that the process of aging which ends in the destruction of the erythrocyte is presumably an intrinsic one and possibly related to exhaustion of enzyme systems with the accumulation of choleglobin within the erythrocyte. Even relatively small amounts of choleglobin cause the red cells to be abnormally fragile *in vitro*. Thus, it would appear that the formation of choleglobin from methemoglobin within the erythrocyte could set in motion the forces leading to removal of the cell from the circulation whether by fragmentation, phagocytosis or hemolysis.

Phagocytosis of whole erythrocytes could be one method of removal and Rous and Robertson⁷⁷ were of the opinion that it does play a significant part in the dog, rat, and guinea pig but not in the cat. Phagocytosis of whole erythrocytes may be seen in peripheral blood in diseases involving the erythrocyte directly. Thus, in feline infectious anemia or haemobartonellosis and in eperythrozoonosis in the ovine, whole red cells have

been observed on rare occasions within mononuclear cells in stained films of peripheral blood.

Isaacs⁷¹ stated that it seems logical to assume that hemolysis plays a part in normal red blood cell destruction, yet there were no direct data demonstrating this. There is ample evidence, of course, in certain diseases involving the red blood cell that its rapid removal from the circulation is by a hemolytic process.⁷⁵

The blood plasma contains a number of substances which are potential lysins⁷⁵ such as fatty acids and soaps which enter the blood stream from the thoracic duct⁷³ in the form of chyle. Plasma also contains proteins and lipids which form complexes with the lytic agents and thereby inhibit the hemolytic action. Cholesterol is said to be one of the more important lytic inhibitors. The normal equilibrium which is established between the natural lysins and inhibitors may become disturbed by massive introduction of lytic agents or by exposure to accelerators. The former situation may occur in dogs fed a high fat diet.⁷⁴ Such diets in dogs are followed by an increase in the excretion of bilirubin. Accelerators are substances which are not in themselves lytic agents but may in the presence of a low lytic-inhibitor blood level release the lytic agent. An example of this is the anemia that sometimes develops in the equine treated with phenothiazine.⁷⁵ It is suggested that this anthelmintic may accelerate the lytic action of the naturally occurring lysolecithin of horse blood.⁶⁷ Ponder⁷⁵ places naphthalene in the accelerator category. Moth balls contain naphthalene and when fed to dogs, a hemolytic anemia may be anticipated.⁷⁸

One of the functions of the spleen is the removal of abnormal red cells as they slowly pass through its sinusoids.⁶⁸ This function becomes particularly prominent in diseases of the erythrocyte *per se*, such as anaplasmosis in the bovine and haemobartonellosis in the feline. In hemolytic diseases the spleen becomes engorged with damaged erythrocytes awaiting final destruction by the phagocytes lining the splenic sinusoids. In certain infectious and neoplastic diseases of man, the red cell membrane becomes altered through adsorption of an autoimmune substance.⁶⁹ This leads to an increase in volume in relation to surface area so that the cell becomes a spherocyte and is readily removed by the spleen.⁷² The designation "acquired hemolytic anemia" is employed and if the condition is associated with a known disease the process is designated as "symptomatic;" if the cause is not known, it is called "idiopathic." A case which was considered to be of the nature of an acquired hemolytic anemia with spherocytosis was observed in a male English Springer Spaniel of one year of age. The anemia developed following an illness of undetermined etiology which began about one month earlier (Appendix, Case 21, p. 356).

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Destruction of Erythrocytes

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Chapter 7

The Erythrocytes in Disease

A COMMONLY occurring finding relative to the erythrocytes in disease is an apparent or relative increase in number due to loss of water from the blood plasma. The water intake of sick animals may not be adequate to maintain water balance and thus, in time, this state of the body is reflected in concentration of the formed elements and chemical constituents of the blood. Erythrocyte number, hemoglobin concentration and PCV are elevated and in a chronic disease in which a secondary anemia has developed, the latter may be masked by the relative polycythemia. An excellent example of this state of affairs is presented in Appendix, Case 2, p. 336. In an acute disease, hemoconcentration may be quite obvious from the blood findings, such as the excessively high erythrocyte number, hemoglobin concentration and PCV (Appendix, Case 1, p. 335).

Polycythemia Vera. A disease in which there is an absolute increase in the number of erythrocytes as well as in the total blood volume. This disease is essentially unreported in animals and in reality true polycythemia is no doubt of rare occurrence in domestic animals. Donovan and Loeb¹ reported on a case of polycythemia rubra vera in the dog and they state that a review of the literature failed to disclose any authenticated reports of the condition in domestic animals. Their patient was a male English Cocker Spaniel, 5-years old, that for several months had shown epistaxis, lethargy, polydipsia, polyphagia, and muscular tremors of the lower jaws. The body temperature was normal but there was a bilateral discharge from the nostrils which was thick and sanguineous. The oral and nasal mucosae had a dark red purplish cast. The laboratory reported: hemoglobin 28.3 grams per cent, PCV 65, WBC 15,700 and RBC 12,000,000.

A case of true polycythemia has been observed in the bovine in our veterinary clinic. This case was a Hereford steer of 8 months of age and from among a group of about 500 head. It was the largest of the group, according to the owner, but was blind and showed a nasal discharge. Close inspection revealed ulceration of the anterior border of the dental pad and encrustations about the nostrils. The mucous membranes were very red due to marked engorgement and congestion of the capillaries. The animal was kept under observation for 8 months and 45 complete blood studies were made. In the third month of observation, 9 liters of blood were removed on each of two separate occasions 1 week apart.

The microhematocrit-PCV was 72.0 per cent on entry; it was 35 per cent on the second day following the removal of the total of 18 liters of blood. The circulating erythrocyte volume gradually increased again reaching 48 per cent at 1 month, 58 per cent at 2 months, and finally 63 per cent by the third month after bleeding. Erythrocyte number was 20 to 24 million per cmm. and hemoglobin concentration was 20 to 23 grams per 100 ml. of blood. The erythrocytes were normocytic normochromic although moderate anisocytosis, poikilocytosis and crenation were constant features. Total leukocyte count usually fell between 12,000 and 18,000 with a mean of 14,200. The Myeloid:Erythroid ratio on two occasions prior to removal of the large volumes of blood was 0.79 and 0.54, respectively. Three weeks after bleeding the M:E ratio was 0.17 and at 6 weeks, it was 0.20. The mean percentages for leukocytes in the 45 differential counts were: mature neutrophils, 30.1; lymphocytes, 55.7; monocytes, 8.7; eosinophils, 4.0; and band neutrophils and basophils not over 1.0. After 8 months the condition of the animal was unchanged and it was destroyed. A careful examination of all organs failed to reveal a basis for the polycythemia.

The Sedimentation Rate of Erythrocytes. The agglutination of erythrocytes in disease is of common occurrence and the degree of red cell clumping may be related to the severity of the disease process. Clumping of erythrocytes enhances their settling-out of the plasma in drawn blood and this is the basis for use of the erythrocyte sedimentation rate in evaluation of a disease. However, the procedure is not of equal value in all domestic species because of certain natural differences in erythrocyte characteristics which in turn significantly influence ESR.

The sedimentation of erythrocytes in their own plasma is dependent upon the propensity for rouleau formation (Fig. 9-4, p. 52), the number of red cells per unit volume of blood, and certain plasma factors, particularly the concentration of fibrinogen and globulin. Among the domestic animals the erythrocytes of ruminants show little natural tendency to form rouleaux in drawn blood and, therefore, there is little or no erythrocyte sedimentation in health and it requires a very serious disturbance of tissue in disease to cause a significant rouleau formation. This statement holds true especially for the cow so that when rouleaux appear in stained films of blood of the bovine such an occurrence should be interpreted as reflecting a grave disease process (Plate V, p. 117). Sedimentation tests are not normally applied to the blood of the cow or sheep because of the almost insignificant settling of red cells even in severe disease. On the other hand, the natural tendency of equine blood to form extreme rouleaux (Plate VII, p. 123) results in such rapid settling of the red cells as to make the test almost useless as a measure of the intensity of a morbid process. The erythrocyte sedimentation test is applicable to the canine. Additional knowledge is needed before ESR

can be properly evaluated in feline and porcine bloods for these species also tend to show excessive rouleaux (Plates IV, p. 113 and VIII, p. 127).

The rate of settling of the erythrocytes is influenced by their number and the size of the cell aggregates. The size of the cell aggregates bears some relation to the concentration of fibrinogen and globulin of the plasma. Before the effect of a pathologic process can be ascertained, the observed sedimentation rate should be corrected for the anticipated sedimentation for any given red cell mass or PCV. The ESR to be anticipated for each PCV unit between 9 and 50 in dog blood is presented in Table 11, p. 139. It should be observed from Table 11 that the greater the PCV, the less becomes the potential for erythrocyte sedimentation. Thus, in disease with hemoconcentration where the PCV is above 50, a failure to obtain an ESR reading after 1 hour in the Wintrobe tube does not necessarily indicate the absence of an inflammatory process or severe tissue derangement (Appendix, Case 1, p. 335). In fact, a sedimentation limited to a few millimeters when the PCV is above 50 per cent should be interpreted as evidence of a grave pathologic process (Appendix, Case 6, p. 340).

Use of the ESR correction table will reveal cases in which the anticipated rate is not reached for a given blood sample. Such an instance is referred to as a negative ESR and such negative results are meaningful. Reticulocytes and young erythrocytes do not readily participate in rouleau formation and therefore when young erythrocytes are present in significant numbers in blood, the observed sedimentation rate is usually less than anticipated. Leptocytes, due to their abnormal shape, do not readily participate in rouleau formation and when many leptocytes are present the sedimentation rate may be less than anticipated (Appendix, Cases 12, 24 and 25).

The ESR is a non-specific test which indicates the existence of an abnormal process much in the same way as elevations in body temperature or abnormal leukocyte counts. A positive ESR indicates the presence of a morbid process. The procedure is useful to detect occult disease and to follow the progress of a chronic disease. The sedimentation rate may be expected to vary from day to day due to the changing composition of the blood as it reflects the disease state. A declining rate suggests healing, an increasing rate suggests that the disease is uncontrolled, and a continuing abnormal rate indicates that recovery has not as yet taken place.

An elevated erythrocyte sedimentation rate is to be anticipated in:

- a. All acute generalized infections.
- b. Localized acute inflammatory diseases of the serous membranes such as the pleura, pericardium, and peritoneum.
- c. Chronic localized infections—the rate varies with the extent and

tissue involved. In formation of an abscess the rate is elevated but as the abscess becomes encapsulated the ESR returns to normal. Inflammation of draining cavities such as the uterus and head sinuses may or may not be associated with an elevated ESR.

- d. Traumatic injury, which includes surgery involving much cutting of tissue, such as ear cropping and enucleation of the eyeball, and extensive burns.
- e. Inflammation of the skin.
- f. Malignant neoplasia.
- g. Pregnancy.

THE ANEMIAS

Anemia is a reduction below normal of the erythrocyte number and/or hemoglobin concentration per unit volume of blood. It is rarely a primary disease but generally reflects a secondary development and, therefore, the unqualified term anemia cannot represent a satisfactory diagnosis for a disease. Anemia in animals is more commonly only one of the results of a generalized disease process and the existence of anemia presents a challenge to the clinician to determine the underlying cause. Treatment is not to be directed at the anemia *per se* except as an emergency measure, for such an approach will result in failure to diagnose and treat the primary disease. The patient, therefore, will fail to receive the benefits of modern veterinary medicine.

Anemia may develop when there is (a) blood loss through hemorrhage or blood sucking parasites; (b) accelerated erythrocyte destruction and/or (c) reduced or defective erythropoiesis.

The clinical signs referable to the anemia are the result of reduced oxygen-carrying capacity of the blood as well as a consequence of certain physiologic adjustments designed to increase the efficiency of the shrunken erythron and reduce the work load of the heart. In hemorrhage, when one-third of the blood volume is lost in a short period, shock occurs and death may ensue. *Tachycardia* or excessive rapidity of the heart and dyspnea or rapid, shallow breathing become prominent signs. In these cases, blood transfusions or plasma expanders are indicated to prevent death. When blood is lost more slowly, as much as 50 per cent may be lost over a 24-hour period without danger to life. The slower loss permits replacement of blood volume by movement of fluids into the circulation from the tissues. In chronically developing anemia, the hemoglobin level may drop to below 50 per cent of the minimum normal without the patient revealing signs of anoxia unless exerted. This is because the body makes certain adjustments to compensate for the lower oxygen-carrying capacity of the blood.

Blood Transfusion. Blood transfusions are used to (a) prevent shock from loss of blood volume in acute hemorrhage, (b) to improve the

oxygen carrying capacity of the blood in order to permit the patient to function more normally, and (*c*) to attempt to restore a normal maturation of erythrocytes in certain "idiopathic" anemias by temporarily giving the erythropoietic tissues a rest.

The mere existence of anemia is not justification for blood transfusion. In the chronically developing anemias, the physiologic adjustments are such that the hemoglobin may drop to 50 per cent of the minimum normal without significant signs of anemia making an appearance. Dogs have entered the clinic with a PCV as low as 5 to 7 per cent (Appendix, Cases 15 and 21); such cases fall into category (*b*). A chronic infection such as described in Appendix, Case 3, where the PCV is 16 per cent, does not represent an emergency need for blood. The dog was too ill to exert itself and for the sedentary existence it was forced to lead, a PCV of 16 was sufficient for maintenance of life. In the canine with chronic interstitial nephritis with uremia, depression of erythrogenesis occurs and a progressive anemia develops. The PCV may fall below 20 but this does not mean that blood transfusion is required or will be helpful. In fact these cases are terminal and the administration of blood is wasteful of the veterinarian's time and the client's financial resources.

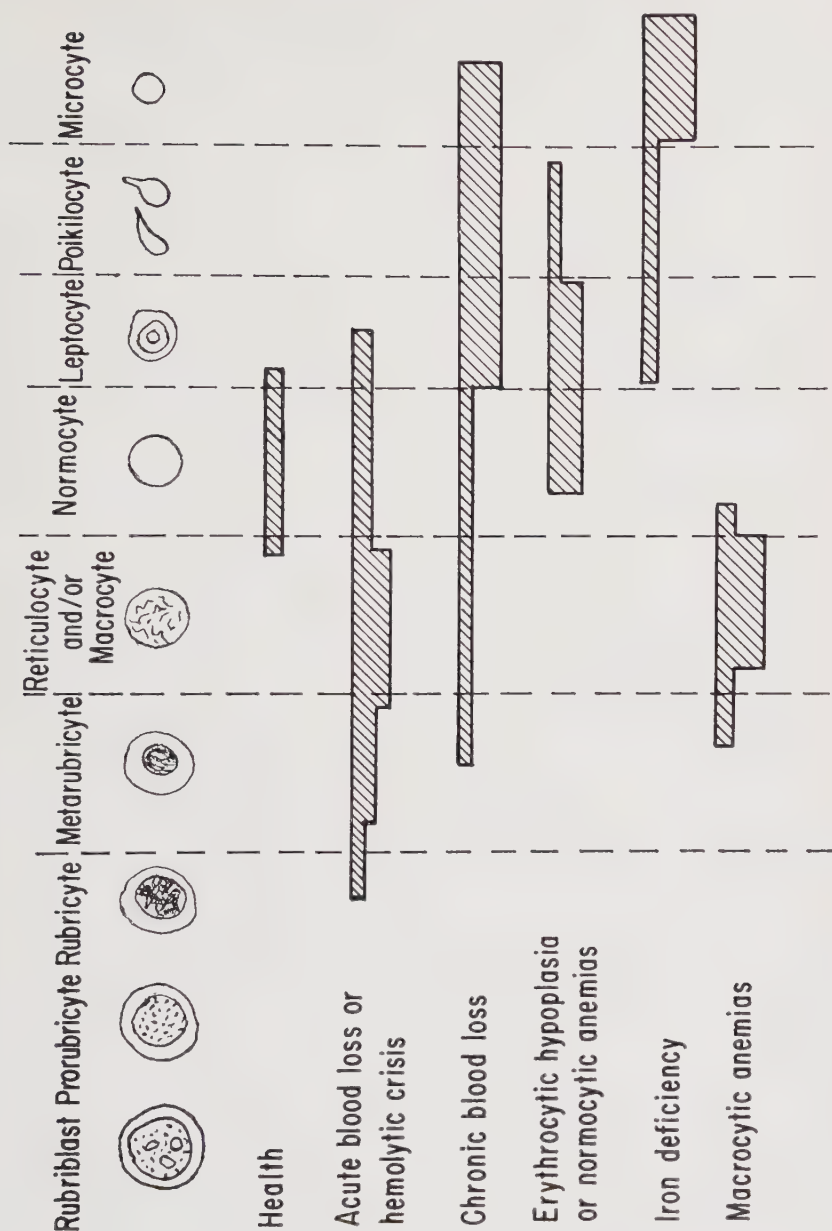
In the case of "idiopathic" anemias in the dog, blood transfusions together with a good hematinic (Appendix, Cases 16 and 21) may have therapeutic value. At least in the present state of knowledge, such treatment can be justified.

In feline infectious anemia blood transfusions are beneficial when hemoglobin level falls below 4 grams/100 ml. of blood and in anaplasmosis in the bovine, blood is definitely indicated when the PCV is below 12 per cent and *there is no evidence of red cell formation*. In the presence of active erythrogenesis in anaplasmosis the decision as to whether or not to give blood should rest on whether or not the cow shows serious respiratory distress. Generally, blood transfusion will not be necessary once evidence of active erythrogenesis makes its appearance in the circulation.

Compensatory Adjustments in Anemia. Heart rate and later heart size are increased to reduce circulation time of the erythrocytes. Blood volume is reduced to bring about a greater concentration of the erythrocytes in the plasma and constriction of peripheral capillaries takes place to reduce the total vascular space. As anemia progresses, plasma protein concentration is decreased thus reducing blood viscosity and work load on the heart.

Clinical Signs in Anemia. In chronic anemia, certain clinical signs may develop as a result of over compensation, namely: (*a*) heart murmur associated with cardiac hypertrophy; (*b*) extreme pallor of the mucous membranes from reduction of blood volume and capillary constriction; and (*c*) edema as result of reduction in plasma protein concentration.

ERYTHROCYTE MORPHOLOGY IN THE VARIOUS TYPES OF ANEMIA



Clinical signs resulting from acute hemolytic anemia are icterus, hemoglobinuria and elevation of body temperature. Icterus without hemoglobinuria occurs when the accelerated destruction of the erythrocytes occurs within the reticuloendothelial system whereas with massive hemolysis of red cells within the blood stream, the free hemoglobin

appears in the urine. The sudden release of hemoglobin and the end products of erythrocyte breakdown leads to pyrexia. Thus, in acute hemolytic disease, a temperature rise should be anticipated and its occurrence does not necessarily reflect the existence of an infectious disease (Appendix, Case 14, p. 347).

MORPHOLOGIC CLASSIFICATION OF THE ANEMIAS AND THEIR TREATMENT

The anemias may be classified on a morphologic basis employing the mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC). The morphologic classification is noncommittal as to etiology but provides a basis for cogitation and the selection of a program of treatment. The size of the erythrocyte in the anemic state is designated macrocytic, normocytic, or microcytic and the mean hemoglobin content is designated either as normochromic or hypochromic. A true hyperchromic state is said not to be possible.

The *macrocytic anemias* may be normochromic or hypochromic and may be divided into transitory and true macrocytic anemias. *Transitory macrocytic anemia* is observed during remission in acute blood loss or acute hemolytic anemia (Appendix, Case 17, p. 353). Reticulocytosis leads to an increase in the MCV but this returns to normal as the emergency is met. *True macrocytic anemia* is associated with maturation arrest in the prorubricyte-basophilic rubricyte stages and leads to a build-up of megaloblastic-type cells in the marrow. Vitamin B₁₂, folic acid, and niacin may be specifically indicated here and should be tried. Blood transfusions may rest the erythropoietic tissue and permit establishment of a more normal maturation sequence²⁸ (Appendix, Case 16, p. 350).

The *normocytic anemias* occur when there is selective depression of erythropoiesis in chronic infectious diseases, malignancies, nephritis with uremia, and in the irradiation syndrome or terminal blood loss in a disease where the marrow is hypoplastic. The normocytic anemias are the usual anemias encountered in animals. Hematinics, B-vitamins, and liver extract injections are not indicated for the erythropoietic tissue is unable to utilize these substances. Blood transfusions, likewise, generally are not indicated for the patient has made the necessary physiologic adjustments to permit it to survive with the anemia. Effort should be directed at diagnosing the primary disease. In protracted cases of the depression anemias, the red cell size may be microcytic but the hemoglobin content is such that the cell is normochromic.

The *microcytic hypochromic anemias* are specific for iron deficiency or failure to utilize iron. Chronic blood loss or iron, copper, and pyridoxine deficiencies must be considered. In severe iron deficiency

anemia, blood transfusions are indicated as an emergency measure and as a means of direct administration of iron (Appendix, Case 15, p. 349).

THE ETIOLOGIC CLASSIFICATION OF THE ANEMIAS

A. *Blood loss anemias*

1. *Chronic bleeding.*

- a. Gastrointestinal lesions. Ulcers, enteritis, coccidiosis.
- b. Neoplasms with bleeding into body cavities.
- c. Vitamin C, K, and prothrombin deficiencies.

2. *Blood sucking parasites.*

- a. Ticks, blood sucking lice, stick-tight fleas.
- b. Hookworm.
- c. Haemonchosis.

3. *Acute bleeding.*

- a. Bracken fern poisoning in cattle.
- b. Sweet clover poisoning in cattle (dicoumarol).
- c. Warfarin poisoning.
- d. Poisoning from trichloroethylene-extracted soybean oil meal (cattle). Also, produced experimentally in the horse and chicken.
- e. Traumatism and surgical procedures.

B. *The hemolytic anemias.*

1. *Infectious types.*

- a. Anaplasmosis; Piroplasmosis.
- b. Haemobartonellosis (feline infectious anemia).
- c. Eperythrozoonosis in sheep and swine.
- d. Leptospirosis in dogs and cattle.
- e. Bacillary hemoglobinuria.
- f. Equine infectious anemia.

2. *Toxic types.*

- a. Chronic copper poisoning in sheep, swine and calves.
- b. Lead poisoning; phenol compounds; benzene and related compounds.
- c. Phenothiazine sensitivity; naphthalene in moth balls.
- d. Parturient hemoglobinuria. (?)
- e. Ricin from castor beans; Snake venom.
- f. Leukemia.
- g. Bacterial toxins: *Micrococcus pyogenes* alpha-toxin in gangrenous mastitis; *Clostridium perfringens* type A-toxin in lambs.

3. *Antigen-antibody reactions.*
 - a. Isoimmunization of newborn foals and swine.
 - b. Acquired hemolytic anemia, symptomatic and idiopathic.
 - c. Incompatible blood in transfusion reactions.
- C. *Secondary anemias (selective depression of erythrogenesis).*
 1. *Organic or tissue disorders.*
 - a. Chronic interstitial nephritis with uremia, especially in the canine.
 - b. Hypothyroidism.
 - c. Malignancies.
 2. *Infectious diseases.*
 - a. All chronic infectious diseases. The decrease in circulating erythrocytes begins to be noticeable after one month of duration of the disease.
 3. *Parasitic diseases.*
 - a. Trichostrongylidosis. The non-blood sucking stomach worms of cattle and sheep may produce a severe anemia.
- D. *Nutritional deficiency anemias.*
 - a. Hypoproteinemia.
 - b. Mineral deficiencies: iron, copper, and cobalt.
 - c. Vitamin deficiencies: B₁₂, folic acid, niacin pyridoxine, thiamin, and riboflavin.
- E. *Hypoplastic or aplastic anemias.* (Granulocytes and thrombocytes are also reduced.)
 - a. Irradiation.
 - b. Bracken fern.
 - c. Trichloroethylene-extracted soy bean meal.
 - d. Sulfonamide sensitivity; chloramphenicol sensitivity.

BLOOD MORPHOLOGY IN THE ANEMIAS (PLATE IX)

Bone marrow response in acute blood loss or destruction:

- a. Thrombocyte number increases immediately to shorten coagulation time and hasten clot retraction.
- b. The leukocyte number increases within hours. This is essentially due to an increase in neutrophilic granulocytes. Later, as reticulocytes are released, immature leukocytes also appear in the blood. The magnitude of the "shift to the left" may be correlated with the intensity of the erythrocytic response (Appendix, Cases 14, 16, 17). In acute hemolytic anemias, leukocytosis and tempera-

ture rise are to be anticipated and should not necessarily be interpreted as evidence of an infectious process.

- c. Immature erythrocytes begin to make their appearance in the circulation in 72–96 hours after the onset of the blood loss or red cell destruction. The peak of release of immature erythrocytes occurs between the fourth and seventh day followed by a gradual decline as the emergency is met. The blood picture during the peak response is characterized by polychromatophilic macrocytes (Plate IX-1) (reticulocytes) nucleated erythrocytes, and erythrocytes with one or more small eccentrically placed dots or nuclear remnants called Howell-Jolly bodies (Plate IX-2). These are about one micron in diameter or about the size of *Anaplasma marginale* bodies (Plate IX-4) or slightly larger in some cases. The variation in size of erythrocytes due to the presence of both macrocytes and normocytes is called *anisocytosis* (Plate IX-1). The MCV is elevated due to the many macrocytes and the MCHC may be below normal. In the cow and sheep, punctate basophilia or basophilic stippling (Plate IX-4) of circulating erythrocytes is a common finding under conditions of intense erythropoiesis (Appendix, Cases 17, 18). Occurrence of nucleated erythrocytes in numbers out of proportion to other evidence of erythropoietic activity is not to be interpreted as a normal response leading to building up of the erythron (Appendix, Case 22, p. 360). Another finding in canine blood that must be properly interpreted is the occurrence of “target cells” during intense erythrocyte production. These “target cells” are reticulocytes and differ from the target cells commonly found in chronic anemia (Plate IX-6) resulting from bone marrow depression.

Signs of Abnormal Erythropoiesis or Bone Marrow Dysplasia. In many chronic diseases, a selective depression of erythropoiesis occurs. The daily removal of over-aged red cells exceeds the ability of the bone marrow to release new erythrocytes. As a consequence, a progressive anemia develops and the body makes physiologic adjustments to render erythrocyte usage more efficient. Thus, the anemia may become well advanced before the owner is aware that a serious condition exists in the animal. The depression anemias are to be anticipated in chronic infections (Appendix, Cases 3, 7), nephritis with uremia in the canine (Appendix, Case 10), malignancies (Appendix, Case 5), hypothyroidism (Appendix, Case 12), and trichostrongylidosis in the sheep and cow (Appendix, Case 19).

In the depression anemias it is common to observe the occurrence of leptocytes. The leptocyte is a thin erythrocyte in which the surface area is increased but with no change in cell volume. This makes it possible for the cell membrane to fold or become distorted and also,

the cell is more resistant to hemolysis by hypotonic saline. Leptocytes show little tendency to participate in rouleau formation or in pathologic clumping of red cells. Thus, in the erythrocyte sedimentation test, the leptocytes tend to fall more slowly and remain scattered throughout the plasma. Failure of leptocytes to settle in ESR in the same manner as normal erythrocytes may lead to a minus value in the corrected sedimentation rate (Appendix, Cases 12, p. 345 and 24, p. 362). When the hematocrit is determined, following erythrocyte sedimentation rate, the leptocytes accumulate in the lower portion of the buffy coat and impart a pink to red color to this area of the buffy coat. Thus, the buffy coat in chronic disease is often partially pigmented due to admixture of leukocytes and leptocytes.

The biconcave erythrocyte of man and the dog is capable of assuming a greater variety of leptocyte patterns than the flat disk red cell which is more typical of the other domestic animals. The leptocyte that is common to all domestic animals and appears in chronic diseases is a *bowl* or *cup-shaped* cell in wet film (Fig. 9-3, p. 52); in the stained blood film it appears to have a sharply "*punched-out*" center while the edges are well filled with hemoglobin (Plate IX-2). It should be pointed out that the bowl-shaped erythrocyte may not always be a true leptocyte (surface area increased in proportion to volume) for at times in acute diseases the majority of erythrocytes present the appearance of having sharply "*punched-out*" centers. It appears probable that under these circumstances the red cell form has become altered in the blood stream. In chronic diseases with leptocytosis the anemia is normocytic normochromic or slightly microcytic normochromic on the basis of MCV and MCHC values.

Legend for Plate IX

1. A, *polychromatophilic macrocyte* or young erythrocyte released from the bone marrow in response to anemia. The variation in erythrocyte size is designated *anisocytosis*.

2. A, macrocyte with a Howell-Jolly body or nuclear remnant; B, the majority of erythrocytes reveal a sharply "*punched-out*" center. This is an artefact resulting from a change in shape of the erythrocyte from biconcave to a cup or bowl-shape. The concavity of the bowl appears as a "*punched-out*" center in the stained blood film. The true shape is seen best in wet film (also see Fig. 9-3B, p. 52).

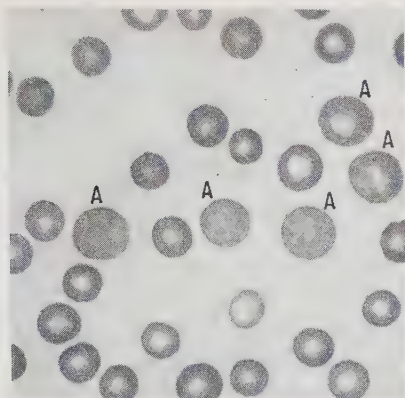
3. The delicate objects on the erythrocytes are *Haemobartonella felis*.

4. A, erythrocyte containing an *Anaplasma marginale*; B, basophilic stippling in a macrocyte—this is of common occurrence in the sheep and cow in active erythro-genesis.

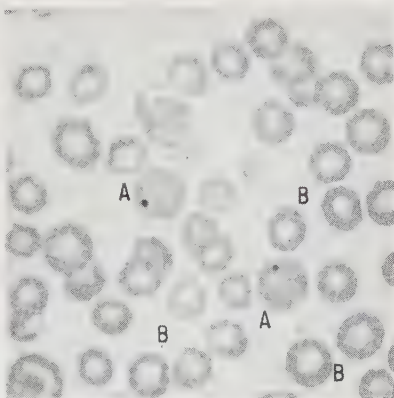
5. Hypochromasia of erythrocytes in chronic blood loss. A, poikilocyte; B, target cell; C, leptocyte with transverse fold.

6. These erythrocytes reveal a characteristic pattern associated with selective depression of erythropoiesis in chronic infection, hypothyroidism, malignancy, and chronic interstitial nephritis. A, target cell leptocyte; B, leptocyte with transverse fold; C, "*bowl-shaped*" erythrocyte presented in a side view.

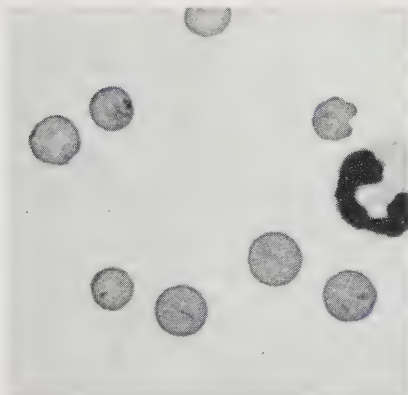
PLATE IX. THE ERYTHROCYTE IN DISEASE



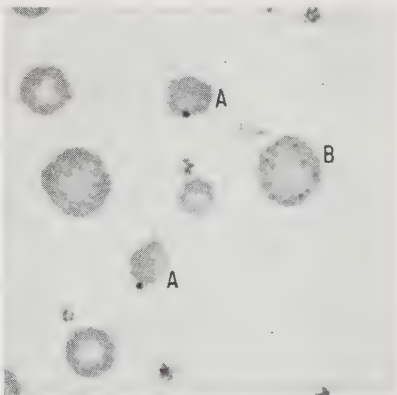
1. Canine blood (600 $\times$)



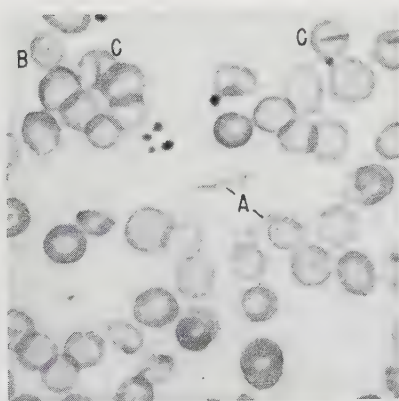
2. Canine blood (600 $\times$)



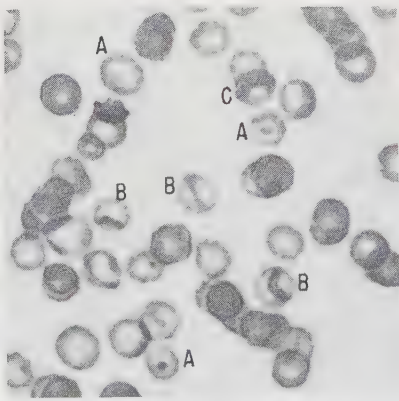
3. Feline blood (900 $\times$)



4. Bovine blood (900 $\times$)



5. Canine blood (600 $\times$)



6. Canine blood (600 $\times$)

(Legend on opposite page)

In addition to the leptocyte, described as cup-shaped or punched-out, the erythrocyte of the dog as well as man presents at least two other possibilities. The "folded" cell (Plate IX-6B) has a raised fold that traverses the center of the cell; in the stained film this cell presents a ring of hemoglobin at the periphery and a bar of hemoglobin extending across the center. The most common leptocyte in canine blood is the "target cell" (Plate IX-6A) and it is also a common finding in human blood. Haden and Evans⁵ were the first to describe this cell in man and they referred to it as a "dimpled" or "Mexican hat" cell. The term "target cell" was applied by Barnett² who also found such cells to have an increased resistance to hypotonic saline. His article contains photographs of models of bowl-shaped cells and target cells. Dameshek⁴ described the typical target cell in stained preparations as, "shaped like a bull's eye, with a peripheral ring of hemoglobin separated by a clear unstained zone from a dense central eye. This characteristic appearance is modified in other cells, in which a bridge of hemoglobin is seen between the peripheral and central masses of hemoglobin."

Leptocytes do not assume the target pattern in the wet film^{2,3} but occur there as bowl or cup cells. Thus, the folding that occurs during preparation of the blood film is an artefact and the number of target cells is found to vary from area to area in a stained film. However, while the target appearance may be an artefact, the red cell is abnormal in that the ratio of surface area to volume is greater than in the normal erythrocyte. *The appearance of target cells or other forms of leptocytes in the absence of frank evidence of active erythropoiesis may be interpreted as an expression of a chronic disease process.*

Target cells have been produced experimentally in dogs and guinea pigs but not in rabbits by removal of the spleen.⁶ In the animals studied before splenectomy target cells were either lacking or present in less than 1.0 per cent and large numbers of target cells were regularly observed after splenectomy in dogs and a considerable number were observed in guinea pigs following splenectomy but not in rabbits. Target cells were also produced by merely ligating the splenic vein. The conclusion was that the spleen either has a direct influence on the red cells passing through it or a hormonal effect on the last stages of erythroblastic maturation in the bone marrow. The life span of the red cell after splenectomy in dog was not increased although leptocytes were present showing increased resistance to hypotonic saline.⁷

ANEMIAS ASSOCIATED WITH NUTRITIONAL DEFICIENCIES

Vitamin Deficiencies. Vitamin B₁₂ and folic acid are essential for erythropoiesis in man. B₁₂ is prepared for absorption from the gut by an intrinsic factor produced in the stomach. When this factor is lacking,

pernicious anemia develops. True pernicious anemia, that is lack of the stomach factor, has not been demonstrated in animals. The removal of $\frac{2}{3}$ of the stomach, the entire duodenum and 30 cm. of the jejunum in dogs failed to result in development of pernicious anemia or a macrocytic anemia.⁹ Vitamin B₁₂ and folic acid deficiencies in man lead to a macrocytic anemia associated with leukopenia and thrombocytopenia. The extent to which these vitamins are required for erythropoiesis in animals or the stage in the maturation series where each may be required is not definitely known. Administration of B₁₂ or liver extract and folic acid to all cases of anemia in animals especially pet animals is without medical foundation. However, in proven cases of true macrocytic anemia the administration of B₁₂ and folic acid may be justified until more knowledge is available. These vitamins are essential in cell multiplication and play an important role in the formation of nucleoproteins. The megaloblast, a cell which develops in the marrow when there is maturation arrest at the prorubricyte stage, is considered to be the result of an abnormality in the metabolism of nucleoprotein.³² Reisner²⁸ states that megaloblasts occur in conditions of severe strain on the bone marrow for red cell production if, at the same time, there is a low level of the maturation factors available. Blood transfusion may cause the megaloblasts of severe anemia to disappear from the marrow by alleviating the demand on the marrow for new blood formation thus permitting normoblastic development to proceed with the available supply of vitamins. The few cases of macrocytic anemia in dogs encountered thus far and treated successfully in our clinic may have responded as much to the blood transfusions as to the hematinic given simultaneously (Appendix, Case 16, p. 350).

Before vitamin B₁₂ was discovered it was common knowledge that an "animal protein factor" was required for adequate growth of some animals, *e.g.*, chickens and swine. This APF factor is now officially defined as vitamin B₁₂ supplement. Baby pigs in which the combined deficiency of B₁₂ and folic acid was produced gave a marked growth response and marked reticulocytosis upon the injection of B₁₂.¹⁹ After 3 weeks on B₁₂ therapy, a second reticulocyte peak was obtained following folic acid supplementation by oral route. Cartwright *et al.*¹³ found growth to be retarded in pigs during the first weeks of life on a vitamin B₁₂ deficient diet. There was no macrocytic anemia and the bone marrow was not megaloblastic. When anemia developed it was normocytic and accompanied by a moderately severe neutropenia. This was in contrast to the production in swine of macrocytic anemia accompanied by leukopenia and slight thrombocytopenia when fed a folic acid deficient ration.¹² Macrocytic normochromic anemia with leukopenia has also been produced experimentally in the rat²¹ with folic acid deficient diets.

Vitamin B₁₂ deficiency develops in cattle and sheep on cobalt deficient

pasture.²⁵ A number of vitamin B₁₂ analogues are produced in the rumen by bacterial fermentation. Cobalt is essential in the molecular structure of vitamin B₁₂ and the term cobalamins has been given to this vitamin and its analogues. Cobalt deficient areas have been reported in Northern Michigan, Northeastern Wisconsin, Florida, British Isles, Australia, New Zealand, and Kenya. The condition produced in cattle or sheep has received various local names such as pine or pining, salt-sickness, bush-sickness, enzootic marasmus, and Nakuruitis. Marston²⁴ describes the cobalt deficiency of cattle and sheep as follows: "The intensity of the symptoms ranges from the acute and fatal malady, through a series of subacute stages, the milder of which affect only the young animals to an incipient state of deficiency which, with change of season may suddenly manifest itself. In sheep it is a wasting disease with profound anemia and failure of appetite. In the early stages, the morphology of the blood cells is suggestive of an aplastic condition of the bone marrow; later as the condition worsens, a profound macrocytic anemia with marked poikilocytosis and polychromasia supervenes. In the acute form of the malady, appetite fails progressively until the animal succumbs in a state of inanition. The syndrome in cattle is not so spectacular; their appetite fails, they lose condition and become anemic."

The ruminants are the source of natural B₁₂ for all meat eating animals; the vitamin is produced by the rumen flora and stored in large quantities in the liver and other glandular organs. Herbivores with a simple stomach and a large cecum such as the horse and rabbit have some B₁₂ production in the cecum.¹⁴ The feces of all animals develop a high level of vitamin B₁₂ with aging as a result of bacterial action. Contamination of the pasture with feces could be the source of B₁₂ for equines. Rabbits may get their supply as a result of habitual coprophagy.

It appears that the dog is able to obtain folic acid from intestinal bacterial action.²² However, when nicotinic acid was restricted the degree of folic acid synthesis was greatly decreased. One case of spontaneous folic acid deficiency has been reported in the dog.⁸ Niacin or nicotinic acid is important to the dog for a deficiency of this vitamin in the presence of a low protein intake leads to development of a syndrome called blacktongue. The symptoms are anorexia, marked dehydration, infection of the oral mucosa accompanied by buccal necrosis and ropy salivation, loss of body weight, and bloody diarrhea. A severe macrocytic anemia^{15,29} with leukopenia results in dogs on experimental nicotinic acid deficient diets.¹⁶ A relationship between tryptophan in the diet and the synthesis of nicotinic acid has been reported. The horse does not require nicotinic acid preformed for it is able to synthesize the vitamin from tryptophan.³⁰ Along this same line it has been shown that niacin is essential for normal erythropoiesis in pigs fed a low protein diet.¹¹ Lack of niacin was associated with development of a moderately

severe, normocytic anemia without leukopenia, but when tryptophan intake was adequate, niacin deficiency did not develop.

Folic acid is obtained in nature from green (foliage) plants. Its molecular structure consists of three parts, namely, (a) pterin = a yellow pigment, (b) para-amino-benzoic acid which is also found in the sulfonamide molecule, and (c) glutamic acid. Its chemical name is pteroyl-glutamic acid or PGA. Folic acid is required for cell multiplication and in the treatment of leukemia, antimetabolites³³ for folic acid have been used to cause the malignant cells to accept a closely related chemical structure but which cannot be utilized by the cell for growth and multiplication. Such a chemical is 4 aminopteryl glutamic acid. In the treatment of bacterial infections with sulfanilamide, leukopenia sometimes developed. This can be explained on the basis of sulfanilamide acting as an antimetabolite for folic acid by virtue of its para-amino-benzoic acid. Folic acid occurs naturally in a conjugated form and before it can be utilized in cell reproduction it must be converted to the active form called folinic acid or citrovorum factor. Vitamin B₁₂, thyroxine, and vitamin C have been credited with playing an important role in the conversion of folic acid to its active form.

Pyridoxine or B₆ was shown to be required by the dog for utilization of iron in the synthesis of hemoglobin.^{15,26} The blood plasma iron is abnormally high and the copper values are at a low normal level during the anemia. The morphologic classification of the anemia is typically that of iron deficiency, namely, *microcytic hypochromic*. Oral administration of pyridoxine in 60 microgram doses per kilo daily was followed in 4 days by reticulocytosis and a return of the erythron to normal. During the anemic phase the MCV was reported to fall below 50 μ . and the MCHC varied from 24 to 28 per cent.

Experimentally, riboflavin deficiency in dogs led to a microcytic hypochromic anemia.³¹ Dogs that were on a riboflavin deficient intake and subjected to phlebotomy, removing up to 25 per cent of the blood volume, failed to regenerate hemoglobin. The dogs could not recover from the induced anemia unless riboflavin was given. Riboflavin deficiency in growing pigs did not lead to an anemia but a marked increase in neutrophilic granulocytes was reported.²⁷

Ascorbic acid or vitamin C is normally synthesized by all animals with the exception of primates, including man, and the guinea pig. The function of vitamin C is said to be the maintenance of intracellular substances of tissues derived from the mesenchyme. Severe deficiency in man results in scurvy which is characterized by hemorrhagic manifestations. It is possible that certain individual animals may not be able to synthesize adequate vitamin C. Two cases that may have had vitamin C deficiency were encountered in dogs in the Veterinary School Clinic (Appendix, Case 24, p. 362). Other functions of vitamin C

mentioned previously are (a) reducing agent for iron in the gut and thereby favoring absorption and (b) participates in the conversion of folic acid to the active folinic acid form.

Mineral Deficiencies. Iron, copper and cobalt are the main minerals that may become deficient in certain animals and lead to anemia. The importance of cobalt to ruminants has been discussed in relation to the synthesis of vitamin B₁₂ by the rumen flora. As far as is known, no other animals are concerned with cobalt deficiency problems.

Copper is a trace element that influences the utilization of iron for hemoglobin synthesis and also stimulates hematopoiesis. In copper deficiency, a microcytic hypochromic anemia very similar to that of iron deficiency is a prominent feature in some animals. In the absence of copper, growth rate is reduced, the hair becomes rough and the color fades before an anemia develops. Copper, unlike iron, is said to be lost continually in the excreta and must be replenished from dietary sources. In England¹⁰ heavy losses from severe anemia due to copper deficiency were reported in pigs 4 to 7 weeks old. Wintrobe *et al.*²³ have reported on experimentally induced copper and iron deficiencies in growing pigs. They concluded that the anemia of copper deficiency in swine is morphologically similar to that due to iron deficiency, namely, microcytic hypochromic. Dogs made anemic as a result of iron deficiency²⁰ had microcytic hypochromic red cells, but the anemia due to copper deficiency in dogs was characterized by normocytic normochromic erythrocytes.

Copper deficiency in grazing ruminants was first shown in 1933 in Holland according to Marston²⁴ and he reports that copper deficient areas are known in Florida, Australia, England, Scotland, Ireland, Norway, Sweden, Schleswig-Holstein, and New Zealand. Copper deficiency in cattle and sheep occurs when such animals are pastured on soil having a high molybdenum content. Molybdenum in excessive amounts interferes with the utilization of copper. Cattle will scour profusely, become debilitated and the pigmentation of the hair fades. Copper administration is used to control the symptoms. Sheep are less susceptible than cattle to excess of molybdenum and equines not at all.²⁴ Present known areas of high molybdenum in California are: Kern, Kings, Fresno and Madera counties, principally on the west side in those areas which were originally overflowed by streams from the Sierra Nevada mountains. In these areas cattle are most affected, with young cattle and calves showing the effects most noticeably. The symptoms are severe scouring, loss of weight and changes in hair coat color. Hereford cattle become dirty yellow and black animals turn mouse-gray; the hair becomes rough and dry. The following is the blood picture in a female Hereford calf of about 6 months of age from a group of about 20 calves affected with molybdenum poisoning. The animal was scouring profusely, was de-

hydrated, and presented a faded hair coat color appearing as dirty yellow. Feces contained 15 ppm. of molybdenum and trichostrongyles were present.

RBC	6,800,000	WBC	12,000
Hb	7.8 grams/100 ml.	Bands	7
PCV	23 per cent	Neutrophils	43
MCV	33.8	Lymphocytes	45
MCHC	33.9	Monocytes	2
		Unclassified	3

Moderate to marked anisocytosis and marked crenation of smaller cells were observed, there were no reticulocytes. The dehydration masked the anemia which was normocytic normochromic.

Iron deficiencies are rare among domestic animals with the exception of baby pig anemia (Table 32, p. 190), animals grazing on iron deficient pastures, and the individual animal heavily infested with blood sucking parasites. Certainly, dogs and cats in the U.S.A., when not infested with blood sucking parasites generally will have adequate stores of iron from their diet. Iron is absorbed from the gut in the bivalent or ferrous form, whereas it occurs in nature in organic complexes in the ferric form. Hydrochloric acid of the stomach releases the iron from the organic complex and reducing substances in the duodenum aid in converting the ferric iron to ferrous iron. Ascorbic acid has been mentioned as a dietary reducing agent. In man, faulty iron absorption results from achlorhydria or lack of HCL in the stomach; this condition apparently is of rare occurrence in animals. A high phosphorus level in the diet ties up the iron so that it is not available for absorption. Conversely, a low phosphorus intake may increase iron absorption.¹⁷

In iron deficiency, hemoglobin production is more deficient than erythrocyte production. Thus, the red cell count is not as markedly reduced as the hemoglobin concentration. In iron deficiency, a maturation arrest takes place in the late rubricyte stage so that while these cells are awaiting hemoglobin synthesis further cell division takes place with the production of definitive red cells that are smaller than normal and deficient in hemoglobin giving rise to a microcytic hypochromic anemia. The bone marrow is actually hyperplastic with a predominance of late rubricytes and metarubricytes (Appendix, Case 25, p. 364).

SOME HEMOLYTIC ANEMIAS WITH HEMOGLOBINURIA

Copper Poisoning. Sheep are especially sensitive to development of hemolytic crisis under conditions of stress when the liver has a high content of copper. Copper available in certain plants or as a result of a contaminant on forage accumulates in the liver and later under a condition of stress the copper is released to the blood stream and brings

about rapid hemolysis of erythrocytes. According to Pearson,⁴⁷ the Australians proved to their satisfaction that the strain of travelling was an important stress factor in promoting hemolytic crisis in sheep, and Pearson comments that Allcroft of England considers starvation after good feeding, exposure to cold, and sudden unaccustomed exercise, to be important predisposing stress factors leading to liver copper release. Hemoglobinuria is an outstanding finding and this is associated with respiratory distress and extreme weakness; the condition is highly fatal. If the individual sheep lives long enough, the sclera becomes icteric and the blood picture is one of marked bone marrow response to acute blood destruction. It has been stated that loss of body weight is one of the principal factors in precipitating a hemolytic crises in sheep that have large stores of copper in the liver. Thus, deaths may increase in number when pastures dry up, unless the loss of body weight is prevented by the use of supplementary feeding.⁴⁹ Hemolytic anemia from copper poisoning has been reported also in swine,⁴² calves,⁵³ and chickens.⁴¹

Post-parturient Hemoglobinuria in Cattle. A disease of high-producing dairy cows, occurring 2 or 3 weeks following calving and characterized by anemia, hemoglobinuria and general weakness, is common in the intermountain areas of the United States.⁴⁴ The visible mucous membranes first become pale and later very icteric and the erythrocyte count may drop to below 2 million per cmm. of blood. The bone marrow responds in typical fashion to acute blood destruction. The MCV is greatly increased during remission of the anemia and basophilic stippling of erythrocytes is common between the fifth and ninth days. The inorganic phosphorus values of the blood plasma are low suggesting a relationship of the disease to phosphorus deficiency.^{44,46,48}

Hemoglobinuria in Calves from Water Intoxication. The author listened to a private discussion among veterinarians attending the Intermountain Veterinary Meeting in January 1957, relative to the relationship of excessive drinking of water to occurrence of hemoglobinuria in calves. A number of practitioners in that area were of the opinion that such was a real possibility. Following this an abstract was observed in the Veterinary Bulletin⁴⁵ relating to the subject and stating as follows: "A condition seen in calves after drinking excessive amounts of water . . . leads to haemolysis and haemoglobinuria . . . associated with cardiac insufficiency and pulmonary edema. The condition is not serious." Also see, Veterinary Record, 73:129, 1961.

Bacillary Hemoglobinuria of Cattle and Sheep (Red Water Disease). This is a specific infectious disease caused by *Clostridium hemolyticum* and characterized by high fever, depression, rapid hemolysis of erythrocytes and hemoglobinuria. The disease is endemic in certain areas of the western states and is most likely to occur in pastures where drainage is poor. The erythrocyte counts may fall below 2.0 million/cmm. of

blood and the hemoglobin may drop as low as 3.5 gm./100 ml.⁵⁰ The disease is often peracute and acute but may be protracted and some animals may recover. If the animal lives long enough for the anemia to enter a stage of remission, the blood picture is typical of a marked response with polychromatophilic macrocytes, basophilic stippling and occurrence of nucleated erythrocytes.

A clinical record of a herd problem diagnosed as bacillary hemoglobinuria was as follows: A rancher in central Nevada lost 30 head of cattle from a herd of 100 between October and early January. Originally the deaths occurred in calves only, but later some older cows died. The course of the disease varied from sudden deaths, to cases which lasted as long as 10 days. Some recoveries were observed. Outstanding clinical signs were temperatures ranging up to 105, nasal discharge, shortness of breath and bloat. Several animals had red colored urine, and in some the nasal discharge was red-tinged. Many cattle went off-feed, became depressed, and stood away from the herd. Others died with no premonitory clinical signs. On January 6, a 2-year-old, Shorthorn heifer was presented for examination. The heifer was in fairly good condition, although quite small for her age. Clinical examination showed paleness of mucous membranes, slight serous nasal discharge, rapid heart rate, and dry, scanty feces, urine normal in color and temperature, 101.5° F.

	Jan. 7 a.m.	Jan. 7 p.m.	Jan. 8 p.m.	Jan. 10 p.m.
RBC	2.18	1.65	1.78	2.26
WBC	27.7/19.9	22.4/14.9	16.8/12.0	17.7/11.3 (corrected for nucleated rbc)
Hb	3.6	3.6	4.3	4.6
PCV	10.0	8.0	13.0	16.0
MCV	46.0	48.0	73.0	71.0
MCHC	36.0	45.0*	33.0	28.7
Icterus index	10.0	7.5	5.0	5.0
Retic.	0.8	2.4	0.1	0.1
Nuc. RBC	39.5/100 WBC	50.0	37.0	55.0

* free hemoglobin in the plasma has produced this elevated MCHC value.

The erythrocyte morphology was as follows:

moderate anisocytosis and
polychromatophilia

marked anisocytosis
and basophilic stippling

This animal recovered and was returned to the owner. A week later a second animal was submitted for necropsy and the diagnosis of bacillary hemoglobinuria was made upon the finding of the typical liver infarct and demonstration of the bacilli in stained liver impression preparations.

Leptospirosis. Hemolytic anemia in the canine and bovine may be caused by leptospira. The clinical signs displayed in leptospirosis are variable but icterus and hemoglobinuria are sufficiently common in the

bovine to be listed among the more common signs of the disease.^{51,52} Leptospirosis in the canine due to *L. canicola* is not commonly associated with a hemolytic process (Appendix, Case 9, p. 343) but when the infection is due to *L. icterohaemorrhagiae* hemolytic crisis with hemoglobinuria may be anticipated. The blood picture in remission is typical of a blood loss disease.

Hemolytic Anemia of the Newborn Due to Isoimmunization. This is a disease that has been reported in mule foals, horse foals and in piglets.^{36,38,39} The dam becomes sensitized to the erythrocyte of the fetus and the antibodies against the red cells of the fetus appear in the colostrum. The young are normal at birth but within hours after suckling colostrum the hemolysis of red cells begins. At first the mucous membranes are pale but later they become icteric. Hemoglobinuria may occur on the second or third day. If death does not take place, the bone marrow response is typical of a blood loss disease. Experiences in Kentucky stables³⁸ indicate that the foal may be expected to live if the erythrocyte count does not fall below 4.0 million or the PCV below 14 per cent. Erythrocyte counts of 3.0 million or less and a PCV of 10 per cent or less are considered critical and a blood transfusion is indicated.

SOME HEMOLYTIC ANEMIAS WITHOUT HEMOGLOBINURIA

Anaplasmosis. This is characteristically a hemolytic disease in which the parasitized erythrocytes (Plate IX-4) are destroyed in the spleen without release of free hemoglobin. Thus, hemoglobinuria is not seen in anaplasmosis. The natural disease may be difficult to diagnose if blood studies are delayed many days beyond the hemolytic crisis. The anaplasma bodies increase in number during the incubation period which varies between 17 and 40 days and during this time the animals are asymptomatic. The parasitized erythrocytes may be removed over a period of a few days and the volume of circulating erythrocytes may drop by 50 to 80 per cent. At the time of greatest fall in erythrocyte number, the body temperature becomes elevated and the animal shows inappetence, depression, and loss of milk in dairy cows. Within 4 to 5 days, anaplasma bodies may be too few in number to permit a certain diagnosis. By this time the anemia is in remission and polychromatophilia, macrocytosis, and basophilic stippling (Plate IX-4) are in evidence. The PCV may go as low as 7 per cent and this is generally unfavorable. However, a PCV of 11 with evidence of anemia in remission need cause little concern. Such an animal has a good prognosis without resorting to blood transfusions if carefully nursed and kept from over exertion.

Changes in the red cell size, red cell volume, and hemoglobin concentration as seen in acute experimental anaplasmosis are shown in data supplied by Dr. J. F. Christensen.³⁵

Note that from the thirtieth to thirty-fifth day the PCV was essentially stationary but the RBC continued to fall. The volume of erythrocytes did not change because the new erythrocytes released from the bone marrow were larger. This is shown by the changes in MCV from day to day (Also, see Appendix, Case 17, p. 353).

Postinoculation Day =	30	31	33	35	39	42	46	66
PCV	13.0	12.0	12.0	12.0	14.0	17.0	18.9	24.0
RBC	3.16	2.58	1.88	1.24	1.41	1.83	2.51	4.88
Hb 	4.4	3.8	3.4	3.4	3.8	4.6	5.2	7.9
MCV	43.0	45.0	72.0	96.7	99.2	94.0	75.5	49.2
MCHC	29.3	31.6	28.0	28.0	27.1	27.0	27.5	33.0

Feline Infectious Anemia. An acute or chronic disease of domestic cats characterized by emaciation, depression, anorexia, high initial temperature, and hemolytic anemia without hemoglobinemia. The name *Haemobartonella felis* has been proposed for the causative organism.⁴⁰ The *Haemobartonella* bodies (Plate IX-3) are inconsistently found on the erythrocytes or free in the plasma. A single negative blood examination is not valid evidence for freedom from infection. It is best to examine the blood at least once daily for 4 days when the disease is suspected and the organism is not found on first examination. The *Haemobartonella* bodies stain rather lightly with the usual Wright's stain and may appear as small, round bodies, small rings or short rods. They appear in the plasma at the height of the infection and may be seen quite well when they cling to the periphery of the erythrocyte. Splitter *et al.*⁵⁴ have described the experimental disease as follows: "There occurred a series of parasitic attacks characterized by an increasing frequency of parasites over a period of several days followed by spontaneous decrease in number often to the point in which blood smears became negative. After an irregular time, usually two to four days or more, a recidivation took place. Three or more relapses, often of increasing intensity, were usually observed before acute anemia developed. These multiple attacks occurred over an average total period of 28 days. Blood values usually decreased most rapidly during and immediately following parasitic relapses and sometimes increased slightly until the next parasitic blood invasion." In acute F.I.A. the erythrocyte count may fall below 2.0 million per cmm. of blood whereas in the more chronic form the red cell count may be somewhat higher, *e.g.*, 3-4

million. The blood film usually shows evidence of erythropoietic response to the anemia. It is important to distinguish between the severe anemia associated with neoplasia of hematopoietic tissues and *Haemobartonella felis* infection. Holzworth⁴³ has prepared a comprehensive discussion of anemias in cats.

Anemia Associated with Hypersplenism. Crosby³⁷ has prepared a review of the normal functions of the spleen. He states that hypersplenism is commonly used to indicate the existence of a pathologic exuberance of splenic function which is manifested by a lack of circulating blood cells. The spleen retains throughout adult life the potential for hematopoiesis but whether or not it has any influence on erythropoiesis in the bone marrow is a topic on which "good friends fall out." Wintrobe in his text on Clinical Hematology states, "Hypersplenism is conceived as being associated with neutropenia, thrombocytopenia or anemia, or with various combinations of these deficiencies in the blood. It is accompanied by a normally cellular or hypercellular bone marrow and usually by splenomegaly." There are situations in human medicine whereby an anemia has been relieved by removal of the spleen. An outstanding example of this is in hereditary spherocytosis. The spleen is responsible for removing the spherocytes from the circulation and following splenectomy the abnormal erythrocytes are able to remain in the circulation for essentially a normal life span.

Hypersplenism has not appeared on the scene in veterinary medicine as a problem to cause any great concern. One case in the canine, namely, Appendix, Case 26, was considered to be representative of primary hypersplenism. Unfortunately bone marrow studies were not conducted on this case and without knowledge of the cellularity of the bone marrow it is not possible to be certain about the pathogenesis of the anemia. It is conjectured that the spleen had taken over the function of hematopoiesis and was doing a very inadequate job of it with the result that the erythrocytes produced by the spleen were unable to survive for a normal life span. Removal of the spleen was followed immediately by an increase in volume of circulating erythrocytes. In 5 days following splenectomy the PCV increased from 24 to 34 per cent and the hemoglobin from 8.8 grams to 11.3 grams/100 ml. of blood.

EXAMPLES OF HYPOPLASTIC ANEMIA

Anemias of this type, also commonly called aplastic, are associated with depression of granulopoiesis and thrombopoiesis. A naturally occurring hypoplastic anemia in cattle is called Bracken Fern Poisoning. Other more artificial conditions that produce similar depression of all cells of the bone marrow are irradiation, antimetabolites, alkylating agents such as nitrogen mustard, and the unidentified factor in trichloroethylene-

extracted soybean oil meal. In all of these hypoplastic anemias, spontaneous hemorrhage due to thrombocytopenia and infection as a result of the granulocytopenia may lead to death of the animal. The anemia is normocytic normochromic for the red cells that remain were produced prior to the bone marrow depression.

Bracken Fern Poisoning. This is a disease of cattle following prolonged consumption of bracken fern when other forage is short. The condition has been produced in sheep experimentally; in the horse the disease takes the form of "staggers." The nature of the specific toxic substance is not known, although there is evidence that it may be an antivitamin called thiaminase.⁵⁹ The toxic substance is destroyed by autoclaving. Under natural conditions, the toxic substance is cumulative and symptoms do not develop until 1 to 3 months after continuous feeding on the fern. The disease is characterized by high temperature, depression, anorexia, bleeding from the body openings, and high mortality. Encouraging results from treatment with DL-batyl alcohol and antibiotics have been reported.⁵⁶ In 1941, it was found that DL-batyl alcohol was a component of a fraction of fat from bone marrow which was effective against granulocytopenia. It was later found that synthetic DL-batyl alcohol plus antibiotics was successful in the treatment of bracken poisoning of cattle if administered before the leukocytes fell below 2,000 per cmm. and platelets below 50-100,000 per cmm. of blood. The mode of action of DL-batyl alcohol on bone marrow is not known.

Trichloroethylene-extracted Soy Bean Oil Meal Poisoning. The clinical picture of this disease in cattle is identical with that of bracken poisoning. In experimental feeding trials, sheep were found to be more resistant than cattle and both the horse and chickens were found susceptible and developed "aplastic" anemia.⁵⁷ In experimental trials with young heifers, the first blood change was noticed on the twenty-fourth day and this was a marked reduction in thrombocytes. Thrombocytopenia was followed by rapid reduction of leukocytes and erythrocytes and death occurred between the thirty-fifth and fifty-sixth day. The neutrophils were reduced to a greater extent than the lymphocytes thus giving a relative lymphocytosis. Bleeding time was prolonged and clot retraction was delayed after the appearance of thrombocytopenia and clot retraction did not occur after the thrombocytes were greatly reduced. Pyrexia, anorexia, weakness and bleeding from the nostrils, mouth, eyes, ears, anus, vulva and prepuce were characteristic signs of the disease. Hematuria rarely was observed, although hemoglobinuria frequently occurred during the last few days of life.⁵⁸

The Radiation Syndrome. The clinical signs in the Beagle following total body radiation may be divided into several periods as follows: (From lecture by A. C. Andersen, A.E.C. Project 4, University of California, Davis.)

Peracute

(a) 1-24 hours

nausea

(b) 1-7 days

Period of well-being

(c) Acute symptoms in

7-21 days

Death

or
survival

anorexia
loss of weight
depression
vomiting
diarrhea

(d) Subacute signs

25-40 days

anemia
infertility

(e) Chronic symptoms

over period of months

obesity
lethargy
decreased efficiency

(f) Latent signs, years later

neoplasia
leukemia
cataract
shortened life

The blood picture shows lymphopenia within hours, the granulocytes tend to show an initial rise during the first 24 hours followed by a gradual decline with marked granulocytopenia between the seventh and twenty-first days. When the total leukocyte count falls below 1,000, few animals survive. Thrombocytopenia plus vascular fragility lead to occurrence of petechial and ecchymotic hemorrhages along the gastrointestinal tract and in the musculature. If the animal survives, the radio-resistant reticulum cells of the bone marrow, spleen, and lymph nodes give rise to hematopoietic development of lymphocytes and granulocytes and these cells begin to return to the peripheral blood after about the fifteenth to twentieth day. At this time, the maturation arrest induced in erythropoiesis begins to show in the development of a normocytic normochromic anemia from which the animal also later recovers.

Death is thought to be due to leukopenia and damage to the gastrointestinal tract which leads to septicemia. By controlling bacterial infection death can be delayed for several days. Animals surviving for 25 days have a good chance to recover from the acute radiation syndrome but later may develop tissue abnormalities as a result of the exposure.

It is of interest that in hibernating animals, such as the marmot, exposed to whole body irradiation, the destructive effects do not become apparent as long as the animal remains in the hibernating state.^{55,60} Immediately upon termination of hibernation, the blood components rapidly diminish in number until the time of death 4–10 days later. This reveals that the rate of development of lethal processes following radiation bears a close relationship to the metabolic rate.

DATA ON IRRADIATED BEAGLE DOGS AT 11TH AND 18TH DAY (from A. C. Andersen)

<i>11th-day post-irradiation</i>	<i>PCV</i>	<i>RBC</i>	<i>Buffy Coat</i>	<i>WBC</i>
Control dog	43	7.7	1.0	11,950
Dog exposed to 300R	35	5.89	0.2	900
Dog exposed to 350R	36	6.1	0.2	1,500 (died)
Dog exposed to 400R	34	4.58	0.2	800 (died)
<i>18th-day post-irradiation</i>				
Dog exposed to 300R	26	—	0.0	—

BLOOD LOSS ANEMIAS ASSOCIATED WITH PARASITISM

A number of blood sucking parasites produce blood loss anemia. In the case of the dog, our clinic has encountered several instances of advanced anemia associated with heavy infestations with the stick-tight flea, *Echidnophaga gallinacea*. Typical of these are Appendix, Cases 15 and 25. The hookworm *Ancylostoma caninum* is a prolific blood sucker and it has been reported that a single worm may remove as much as 0.8 ml. of blood in a 24-hour period.⁶¹ In acute hookworm disease the blood picture is one of acute blood loss, whereas in the more chronic disease the anemia may be microcytic hypochromic. Heavy infestations with the stomach worm *Haemonchus contortus* in sheep present a blood picture of acute blood loss (Appendix, Case 18, p. 353); however, in flock problems of Haemonchosis the anemia may develop more slowly and become well advanced with little or no evidence in the blood of reticulocytosis. A report of anemia in cattle caused by the blood-sucking louse, *Haematopinus eurysternus*,⁶³ states that the condition of the animals became critical when the PCV decreased to 13 per cent and that a PCV as low as 9.0 per cent was observed. Removal of the lice was followed by a prompt recovery from the anemia.

Heavy infestations with coccidia will lead to bloody diarrhea and anemia. In 9 dogs artificially infected with coccidia, the average erythrocyte count fell from a normal of 6.3 million to 3.4 million per cmm. of blood in 6 days and then returned to the normal level over the next 11 days.⁶²

SECONDARY ANEMIAS FROM ERYTHROCYTIC HYPOPLASIA (PLATE IX-6)

Chronic Infections. Normocytic normochromic anemia is commonly found in association with chronic infections. Even a sterile abscess produced in the dog by injection of 1.0 ml. of turpentine subcutaneously will diminish the production of new hemoglobin in the anemic dog.⁶⁹ Observations upon porphyrin excretion show an increased excretion of coproporphyrin I and III thereby indicating some abnormality of hemoglobin synthesis associated with the chronic septic process.⁷¹ The blood plasma is low in iron (hypoferremia) and this is accompanied by a hypercupremia as well as an increase in erythrocyte protoporphyrin and coproporphyrinuria.⁶⁶ On the basis of the latter findings a working hypothesis was suggested as follows: As a result of the inflammatory reaction, iron is diverted to the tissues and is not available for hemoglobin synthesis. This is probably because of the persistent and urgent demand for iron to fulfill some function in relation to infection which has a greater priority for iron than hemoglobin formation. The anemia cannot be corrected by the administration of iron.⁶⁵ The anemia of chronic infection is documented in Appendix Cases 2, 3, and 7.

Nephritis with Uremia. Advanced chronic interstitial nephritis in the dog is generally associated with a normocytic normochromic anemia. A similar situation occurs in man when uremia is present in chronic glomerular nephritis; the degree of anemia in man has been reported to parallel the degree of nitrogen retention.⁷⁰ Bone marrow studies in man have revealed definite evidence of hypoplasia of the erythropoietic tissues when the non-protein nitrogen level of the blood was over 150 mg. per cent.⁶⁴ Levin and Gregory⁶⁸ found normal or hypercellularity of bone marrows in 50 per cent of human cases of uremia and they suggested hemolysis and functional impairment of erythropoiesis as possible factors responsible for the anemia. Erslev⁶⁷ experimentally produced uremia in rabbits and made observations on the erythropoietic factor. He concluded that severe uremia is associated with significant suppression of erythropoietic activity of serum and depression of metabolism of erythropoietic cells. Appendix, Case 10 is typical of the depression anemia associated with nephritis and uremia in the canine.

Neoplasia. Neoplasia, especially when malignant, causes a selective depression of erythropoiesis but stimulates a granulocytosis. When blood loss from the neoplasm is superimposed, the blood picture will be a combination of signs of depression of erythropoiesis (leptocytes) and reticulocytosis (polychromatophilia).

In neoplasia of the hematopoietic tissues, with or without leukemia, anemia is encountered in the dog and cat but it is of variable occurrence in the cow. In the dog, anemia associated with lymphocytic

leukemia is generally of moderate degree with the PCV in the 20–30 per cent range. In the cat, severe anemia is commonly encountered in neoplasia involving the hematopoietic tissues; the erythrocyte number may fall below 2 million per cmm. of blood. The anemia of the leukemia-complex is normocytic normochromic and there is generally an accompanying but variable reticulocytosis. The anemia may be the result in part from invasion of the bone marrow by neoplastic cells and in part from reduced life span of the erythrocytes due to some intrinsic defect in the red cell or to some extracorporeal factor producing hemolysis.³⁴

Hypothyroidism in the canine. This anemia is commonly borderline with the PCV ranging between 30–37 per cent, although a few cases have fallen below 30 per cent and in other instances the PCV remained in the normal range. The anemia is normocytic normochromic with leptocytosis a prominent feature (Appendix, Case 12, p. 345).

Parasitism in Sheep and Cattle. The trichostrongyloid parasites of cattle and sheep (excluding *Haemonchus contortus*) may produce a severe normocytic normochromic anemia that has all the appearances of selective depression of erythropoiesis (Appendix, Case 19, p. 354). The PCV may fall to 12 per cent and there is no evidence of reticulocytosis to compensate for the anemia. A PCV as low as 5 per cent was observed in a lamb in association with a very heavy parasitism of this nature.

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Chapter 8

The Leukocytes

THE term leukocyte includes all white blood cells and their precursors. The leukocytes use the blood as a means of transport from their site of origin to their destination in the various tissues of the body.

The total leukocyte count, stated in number of cells per cubic millimeter of peripheral blood, is a reflection of the need for leukocytic functions in the various tissues of the body. The total leukocyte count in health is given as a range which takes into account the influence of moderate activity, such as walking and eating, on the number of leukocytes in active circulation. Muscular activity with an increase in heart rate and respiration brings about an elevation in the number of circulating leukocytes; this is referred to as "physiologic leukocytosis" and is explained on the basis of a redistribution of leukocytes that had become sequestered in collapsed capillary beds during periods of relative inactivity. The effect of strenuous muscular activity on total leukocyte count in the horse is shown in Table 35, p. 213.

The regulation of peripheral leukocyte level has been investigated by Ambrus and Ambrus¹ by means of transfusing labeled leukocytes into the dog. The transfused cells disappeared from the circulation in 2-3 hours and upon the injection of epinephrine, labeled leukocytes reappeared in the blood. The main source of leukocyte release was said to be the pulmonary and splenic circulations and to a lesser degree, the splanchnic circulation. Transfused labeled granulocytes appeared to migrate to the capillary walls in the pulmonary circulation and then they were either released again to circulate in the blood or they migrated into the lung alveoli and later appeared in the saliva. Transfused labeled lymphocytes migrated through the walls of the small intestine and were eliminated. The investigators suggested that the continuously high level of elimination of leukocytes through the respiratory and gastrointestinal passages may be a mechanism to protect these organs against infection. Bierman² states that there appears to be a constant ebb and flow of leukocytes moving into and from the pulmonary circulation and this movement is reflected peripherally as a fluctuating leukocyte count. The liver is an important leukocyte removal site but it is not as active as the lungs in this respect and likewise the spleen is a potent leukocyte sequestration site.

BRIEF SUMMARY OF THE ORIGIN AND FUNCTIONS OF LEUKOCYTES

The neutrophils, eosinophils, and basophils originate in the bone marrow from a multipotent stem cell called a myeloblast. Taken together they are called granulocytes and the specific name of each type refers to the staining characteristic of their cytoplasmic granules. However, the neutrophils of the various domestic animals reveal such a marked variation in staining characteristics that the term heterophil has been suggested as a more suitable term for this cell of the granulocytic series. The lymphocyte originates in the thymus, spleen, lymph nodes and other widely scattered lymphocytic foci throughout the body. The origin of the monocyte is debatable; one group holds it to be a product of the widely disseminated reticuloendothelial system, while another group considers the monocyte to be a special cell of the lymphocytic series.

Neutrophils, also called microphages, are active in the initial stages of inflammation and destroy the coccal forms of bacteria by phagocytosis. In severe infections, immature forms of neutrophils appear in peripheral blood but forms more immature than the metamyelocyte are incapable of participating in phagocytosis of antigen. Death of neutrophilic leukocytes in peripheral blood may lead to marked clumping, perhaps, as a result of random collision and the inherent stickiness of the cells. Sieracki²⁰ has presented an excellent review of the neutrophil leukocyte accompanied by 195 references.

Eosinophils are assigned the function of detoxification and it has been suggested that they inactivate histamine or histamin-like toxic materials.²³ They are found in the tissues at the portal of entry of foreign materials, e.g., the gut wall, subcutis, and the lining of the respiratory tract. The eosinophils are attracted to the site of antigen-antibody reaction. Speirs²¹ states that antigenic material *per se* does not produce a systemic increase and induces only a slight local accumulation of eosinophils. However, after an animal has been sensitized, the injection of antigen will produce an intense accumulation of eosinophils at the site of injection. The eosinophil disappears from the circulation during stress. This is seen in the differential leukocyte count of early bacterial infection and the eosinophil returns to the blood during convalescence. The eosinophils increase in situations involving the decomposition of body protein and this may reflect a function of detoxification.

Basophils. The function of this granulocyte is even more elusive than that of the eosinophils. It is probable that the basophil shares its function with the tissue mast cell. The granules contain or consist of heparin and this suggests an anticoagulant activity. Fredricks and Maloney⁹ postulate that the basophil, like the mast cell, is a "heparino-

cyte" which releases its anticoagulant material in areas of inflammation in order to prevent clotting and stasis of blood and lymph. The peritoneal cavity contains tissue mast cells. Chronic bleeding into the cavity is not followed by clotting of the blood. This is no doubt a protective mechanism for clotting of blood would lead to formation of adhesions between intestinal loops as well as between the intestine and the peritoneum proper. The prevention of blood clotting in the abdominal cavity may be a function of the mast cells.

Lymphocytes show a relative or absolute decrease during the initial phases of infection and return during the period of convalescence. The lymphocytes have been attributed a role along with the plasma cell in antibody formation.

Monocytes are the macrophages and their special enzyme systems are called upon to handle the more difficult pathogens especially those causing a granulomatous inflammatory response. In this category may be placed fungi, protozoa, tubercle bacillus and the brucella species. The monocytes are commonly numerous in a chronic infectious process where there is much tissue debris to be removed (Appendix, Cases 2, 3, and 4).

HORMONAL INFLUENCE ON LEUKOCYTES

It is now an accepted fact that under conditions of stress the adrenal cortical secretion depresses the number of circulating eosinophils and lymphocytes and leads to an elevation in number of circulating neutrophils. A review of the literature on this subject to 1955 has been presented by Gordon.¹⁰

Dougherty and White⁷ showed the relationship of ACTH to the stress phenomenon by the injection of purified pituitary adrenotrophic hormone into normal and adrenalectomized mice, rats, and rabbits. In normal animals, the injection of ACTH was followed within the hour by neutrophilia and lymphopenia with a maximum lymphopenic effect at 9 hours followed by a return to normal. Similar injections into adrenalectomized animals was not followed by a change in the differential leukocyte count. Dougherty and White⁸ observed that the lymphopenia was the direct result of dissolution of lymphocytes with release of cytoplasm to the lymph. On the other hand, no direct lytic effect of corticoids upon circulating eosinophils has been demonstrated. Archer² injected hydrocortisone and ACTH into the horse in doses sufficient to produce peripheral eosinopenia. Studies of bone marrow revealed no change in the eosinophil content and after 12 hours an eosinophilia was observed first in the bone marrow and later in the circulating blood.

The use of circulating eosinophil counts for evaluating functional activity of the adrenal cortex by injection of ACTH has been described in Chapter 2.

The triad of neutrophilia, eosinopenia and lymphopenia following stressful stimuli is commonly seen in the stress of disease and may be anticipated in bacterial infections, traumatic injury, extensive burns, malignancy, severe hemorrhage or blood destruction, and at parturition.

EFFECTIVENESS OF GRANULOPOIESIS IN DISEASE

The ability of the bone marrow to respond to a bacterial infection is to be measured by the *magnitude of the total leukocyte count* and the intensity of the response is to be gauged by the *extent of left shift*. The severity of the infection is measured by the occurrence of toxic changes in the neutrophils. The following are recognized as manifestations of toxicity:

- a. Diffuse basophilia of the cytoplasm. This characteristic which relates to the staining of the cytoplasm in neutrophils, may be seen in all domestic animals under conditions of extreme toxicity.
- b. Occurrence of blue-black granules, from few to many, in the cytoplasm is referred to as toxic granulation. This form of toxic manifestation has been seen in the sheep and cow but not in the dog and horse.
- c. The cat may present a few angular dark bodies in the cytoplasm of some neutrophils under conditions of health (Plate IV, p. 113). The number of such bodies may be seen to increase in disease with leukocytosis and left shift and may represent toxicity.
- d. A common manifestation of toxicity in neutrophils of the dog and cat is the occurrence of vacuoles located along the periphery of the cell so as to suggest a moth-eaten appearance.

SPECIES VARIATIONS IN TOTAL LEUKOCYTE RESPONSE IN DISEASE

- a. The dog and cat respond dramatically to infection; counts of 30,000+ are common and total counts of 50,000 to 100,000 are not rare. (Appendix, Cases 2, 3, and 4.)
- b. In the pig, total leukocyte counts of 20,000 to 40,000 are not uncommon.
- c. In the horse, the general level of leukocyte response to infection is in the range of 15,000 to 25,000. Marked leukocytosis in the horse is 25,000–30,000 whereas extreme counts are in the range of 35,000+. (Appendix, Cases 5 and 7.)
- d. The cow is even less responsive than the horse to infection. Very often the total count remains within the normal range of 4,000 to 12,000 but there is a neutrophilia of 50+ per cent and perhaps a

left shift. A marked leukocytosis would be counts of 15,000 to 25,000 and extreme leukocytosis would be counts above 25,000. Total counts of 40–50 thousand have been recorded in infections in the cow but such counts are very rare. In the cow a neutrophilia above 50 per cent and/or occurrence of band forms in excess of 2.0 per cent are indicative of the stress phenomenon. (Appendix, Cases 8, 27 and Table 56, p. 327.) A partial explanation can be found in the normal differential leukocyte count of the cow and also in the sheep and goat. The two cell types, eosinophils and lymphocytes, which are depressed by adrenocortical activity in stress, constitute 65 to 70 per cent of the circulating leukocytes and the neutrophils which increase under the stimulus of stress represent less than 30 per cent. Thus, initially, the fall in number of circulating eosinophils and lymphocytes may exceed the increase in number of neutrophils with the effect of an actual reduction in total leukocyte count as neutrophils move from the blood into the affected tissues.

CLASSIFICATION OF THE TOTAL AND DIFFERENTIAL LEUKOCYTE RESPONSE

- a. *Regenerative Left Shift.* This shift is characterized by a leukocytosis due to neutrophilia and with the appearance of immature neutrophilic granulocytes in peripheral blood. A slight shift to the left is limited to the occurrence of band neutrophils. A moderate left shift includes both band and metamyelocyte neutrophils while a marked left shift would bring myelocytes and progranulocytes into peripheral blood (Appendix, Cases 2, 3, 4, and 7).
- b. *Degenerative Left Shift.* The total leukocyte count remains within the normal range or is only slightly elevated, while the occurrence of young granulocytes in the circulation is prominent. It reflects inability of the bone marrow to rise to the occasion and put forth a large number of mature cells. A degenerative left shift is common in septicemia (Appendix, Case 1).
- c. *Leukemoid Blood Picture.* Similar to a regenerative left shift. The hemogram suggests granulocytic leukemia in that there is a significantly elevated total count with left shift but the process stimulating the count is other than leukemia. A leukemoid hemogram is to be anticipated in pyometra in the canine (when the cervix is closed with retention of pus), abscess formation, and following severe hemorrhage or hemolytic crisis (Appendix, Cases 4, 7, and 27).
- d. *Absolute and Relative Leukocyte Numbers.* When the differential leukocyte count is expressed in percentage it is necessary to scrutinize each percentage figure in light of the total leukocyte count to

determine whether a normal number of cells exists or an absolute increase or decrease has occurred. A good example is the lymphocyte percentage in Appendix Case 2. The normal lymphocyte range stated in percentage for the canine is 12–30; this, however, is based on a normal total leukocyte count. In Case 2, the lymphocytes represent 2 per cent of the differential leukocyte count. This is a *relative lymphopenia* for 2 per cent of a total count of 100,500 is 2,010 lymphocytes and the mean lymphocyte distribution in the normal dog is 2,200 per cmm. (Table 8, p. 131). Another way to arrive at a *relative* or *absolute* status of a given cell type is to recall the normal mean total leukocyte count for the species and the percentage range for the cell in question. In the dog, the lymphocyte percentage range is 12–30 and the mean total leukocyte count is 11,000. Given a total count of 100,000, one readily determines that such a count is 9 times the normal mean and 9 times the lymphocyte percentage figure of 2 equals 18 per cent which is within the normal range.

If the percentage distribution of a cell in the differential leukocyte count is abnormal, then the situation must be described as being *relative* or *absolute*. On the other hand, if the percentage distribution is normal for any cell type but the total count is either high or low, it is only necessary to affix the suffix -cytosis, -philia, or -penia as the case may be. As an example, see Appendix, Case 2, p. 336, under date of September 3, where the 4 per cent monocytes falls within the stated normal range for the dog. However, in the face of a 51,000 total leukocyte count, the absolute count of monocytes is 2,040 which exceeds the upper limit of normal and therefore the 4 per cent monocytes must be described as a monocytosis (Table 8, p. 131).

LEUKOCYTE RESPONSE IN RELATION TO THE TYPE OF DISEASE PROCESS

- a. In bacterial infection with septicemia there is often no leukocytosis but more commonly a degenerative left shift (Appendix, Case 1).
- b. Bacterial infection with localization and pus formation stimulates a marked neutrophilia. Before the abscess becomes encapsulated the total count may be marked to extreme (Appendix, Cases 2, 3, 4, 7 and 8).
- c. Neutrophilia is associated with a variety of non-infectious conditions, which stimulate the stress reaction, such as malignancy (Appendix, Cases 5 and 6); chemical intoxication (Appendix, Case 22); metabolic intoxication such as uremia (Appendix, Case 11 and Chapter 10, Table 48); the post-operative state; and hemolytic

crisis or significant blood loss (Appendix, Cases 13, 14, 17, 24, and 25).

- d.* Eosinophilia is most commonly associated with antigen-antibody reactions.
- e.* Monocytosis is evidence of chronicity, reflecting the need for destruction of pathogens too difficult for the neutrophils to handle and/or the need for the removal of tissue debris in the chronic inflammatory process.
- f.* Leukopenia is common to most diseases caused by viruses.

INTERPRETATION OF THE LEUKOCYTE PICTURE

- a.* Neutrophilia with slight left shift and with persistence of eosinophils suggests a mild infection.
- b.* Neutrophilia with a relative lymphopenia and an absolute eosinopenia indicate a moderately severe to severe infection (Appendix, Case 9, p. 343).
- c.* A marked absolute reduction in lymphocytes which persists justifies a poor prognosis (Appendix, Cases 5, 6, 7, 8, 10, and 11).
- d.* An extremely high leukocyte count consisting mainly of neutrophils is considered to be an unfavorable sign (Appendix, Cases 3, 4 and 7).
- e.* Immature neutrophils in excess of mature neutrophils is unfavorable; this rule does not appear to apply to the bovine in the initial response to infection.
- f.* A severe infection is indicated when toxic changes in neutrophils are a prominent feature. Prognosis should be guarded.
- g.* A falling total count with a reduction in neutrophils and a return of lymphocytes and eosinophils represent the changes in convalescence.
- h.* A leukopenia is common to virus diseases but is also present in overwhelming infections. Leukopenia is commonly observed in the early stages of acute mastitis and other similarly acute bacterial infections in the bovine (Table 57, p. 329).
- i.* Eosinophilia is difficult of interpretation but should be related to the possibility of an allergic response or detoxification in decomposition of body protein.
- j.* Monocytosis reflects chronicity.

DYNAMICS OF GRANULOPOIESIS

The lifespan of the granulocyte leukocyte covers the intramedullary developmental phase, the intravascular phase, and the tissue phase. The leukocytes use the blood stream mainly as a means of transport between their points of origin and destination in the tissues. Thus the

number of leukocytes in peripheral blood is a measure of the body's need for leukocyte function in the tissues. The life span is not easily determined for the leukocyte spends a portion of its existence in the tissues and this latter phase cannot be measured with methods now at hand. More accurate life span data are becoming available through use of radioactive phosphorus, P^{32} . This isotope is incorporated into the leukocyte desoxyribose nucleic acid only during the intermitotic phase and is not exchanged subsequent to cell division. Using P^{32} on the human granulocyte, Kline and Clifton¹⁴ found that 4 days were spent at the site of formation and 9.2 days in the circulation. Patt and Maloney¹⁷ investigated the maturation time of the neutrophil granulocyte by radioautographic analyses of marrow and blood from dogs receiving tritiated thymidine. The labeled nucleoside is incorporated into the desoxyribonucleic acid (DNA). Since approximately half of the labeled DNA remains in the daughter cells of a labeled progenitor, several mitotic cycles may take place before the label is no longer detectable. About 15 to 20 per cent of myelocytes became labeled within 2 hours after injection of tritiated thymidine. Densely labeled myelocyte telophases were observed in 4 hours, labeled metamyelocytes appeared in the marrow within 12 to 24 hours, labeled band cells in 24 to 36 hours and labeled segmented neutrophils were detected in aspirated marrow within 2 to 3 days and labeled segmenters were present in peripheral blood in 3 to 4 days after the injection of tritiated thymidine. The peak of labeled cells in the circulation, 25 to 30 per cent, was reached in 4 to 5 days after initial labeling of the myelocytes of the bone marrow.

The question arises as to whether there is a leukocyte "pool" which can be drawn upon as the need arises or whether the supply to each demand is dependent upon a direct stimulation of the bone marrow for immediate production of new cells. If a mass of non-circulating leukocytes exists, such a "pool" of cells would be in equilibrium with the leukocytes in circulation. To determine the response to the rapid removal of leukocytes from the circulation, Craddock *et al.*^{5,6,18} developed a technic called leukopheresis. The method as applied to the anesthetized dog consisted of removing the leukocytes from $1\frac{1}{2}$ blood volumes every hour for 3 hours. This was accomplished by repeatedly removing 40 to 50 ml. of blood and depleting the leukocytes by several different technics and reinjecting the blood. Leukopheresis produced a leukopenia in 15 of 17 dogs (2,000 or less per cmm. blood) and after a delay of 30 to 90 minutes, the leukocyte count started up and in $4\frac{1}{2}$ hours the cells were back to 100 per cent value. The count continued to rise over the next 24 to 36 hours reaching 150–200 per cent of the baseline. Repeated leukopheresis after the leukocyte count had reached its maximum was accompanied by a progressive shortening of the delay period before leukocytosis began. Both normal and irradiated dogs could

rapidly replace the number of leukocytes circulating at any one time. The irradiated animal showed the more delayed replenishment and if repeatedly leukopheresed it was unable to respond due to depletion of leukocyte stores. In the normal dogs there was no change indicative of increasing immaturity of the marrow or circulating leukocytes until the third or fourth day. This suggested that the initial response involved release of preformed leukocytes from storage areas and, therefore, the initial response did not depend upon an immediate production of cells by the bone marrow. Labeling of granulocyte leukocytes during formation in the bone marrow through the use of P^{32} led to the conclusion that most of the leukocytes were released from reserves in the marrow rather than from tissue stores. In fact it was doubted whether granulocyte leukocytes are available to return to the blood stream once having entered the tissues. The authors concluded that the release of cells from the marrow is geared to the utilization rate of leukocytes in the tissues. A persistently low neutrophil count, despite obvious stimulation to neutrophilic leukocytosis, indicates a depleted marrow reserve, as in leukopenia of overwhelming pyogenic infection. A leukocyte reserve is necessary to prevent the occurrence of marrow depletion by the sudden imposition of a greatly increased rate of peripheral utilization of leukocytes. The marrow reserve affords a huge buffer between supply by growth, which is fixed within certain limits, and demand, which may vary widely. These studies on leukopheresis have demonstrated that the normal dog has the capacity of supplying a greatly increased peripheral demand for leukocytes. Also, it can do so repeatedly without development of an "exhaustion" picture. Based on a quantitative study of the cells of the granulocytic series in the bone marrow of young albino rats, Kindred¹³ came to the conclusion that the bone marrow of the rat can supply 58 times the average need per hour for neutrophils. Osgood¹⁶ has estimated the ratios of circulating to non-circulating leukocytes in man to be as follows: granulocytes, 1:60; lymphocytes, 1:400; and total leukocytes, 1:200.

THE BIOLOGY OF INFLAMMATION

Menkin¹⁵ has conducted extensive studies on the mechanism of inflammation and a brief summary of his observations follows. Inflammation is a manifestation of severe cellular injury, and as such, it represents the basis of all infectious processes. The injured cells produce a number of chemical substances that initiate and perpetuate the inflammatory reaction. Initially the reaction is on the alkaline side and a substance called *leukotaxine* acts by increasing capillary permeability and serves as a chemotactic agent to draw the neutrophil leukocytes into the area. Leukotaxine is unable to stimulate granulopoiesis but this is accom-

plished by a thermolabile leukocytosis-promoting factor (LPF) also produced by injured cells and present in the alkaline exudate. LPF is absent in normal blood serum, but it can be recovered from the serum of an animal with a concomitant inflammation. This fact suggests that it stimulates the bone marrow *via* the blood stream. The initial cells to infiltrate the area of injury are the neutrophil leukocytes and these are subsequently replaced by macrophages. With some irritants the neutrophil leukocyte phase may be lengthened and the macrophage phases shortened—for example, a staphylococcus abscess, or, vice versa, a tubercle. The neutrophil leukocytes are unable to survive at a pH below 7.0 whereas mononuclear phagocytes appear fairly normal at a pH 6.9 to 6.8. At a somewhat lower pH all types of leukocytes tend to be injured. The result is a state of suppuration or pus formation. The lymphocytes appear to be unaffected, at least directly, by changes in hydrogen-ion concentration. As the reaction becomes acid, new substances of cellular origin come into play to continue the inflammatory process. *Exudin* takes the place of *leukotaxine* and a thermostable leukocytosis promoting factor appears along with exudin. Other substances of injured cell origin are designated *necrosin*, *leukopenin* and *pyrexin*. The terminology employed explains the principle action. Necrosin is of special interest for it can induce an acute inflammation and thus is capable *per se* in reproducing the effects seen in inflammation. Necrosin can at times be recovered from the blood serum of an animal with an acute inflammation. This may explain the toxic effect of a lesion on organs at a distance. The liver and kidney appear especially susceptible to injury upon injection of necrosin. Liver cells become swollen and laden with fat or the cells may lose their outline and liver cords become stippled with coarse granules. The epithelial lining of the kidney tubules may show irregularities and vacuolation with foci of leukocytic infiltration. Necrosin has a leukopenic effect and is found mainly in the acid exudate; also, a substance called leukopenin can be recovered from the alkaline exudate of the initial stages of inflammation. Thus, leukopenin may be responsible for initial leukopenia which is occasionally seen in some infectious diseases and necrosin may cause the fall in circulating leukocytes during the acid or later stages of inflammation.

The anti-inflammatory action of the corticosteroids has been shown to be due to interference with activity of injured cells in producing the sequence of chemical substances responsible for the inflammatory process. Adrenal cortical extract, cortisone or compound F inhibit leukotaxine but ACTH has no direct action on it. On the other hand, ACTH inhibits exudin by direct action but cortisone is ineffective. Menkin concludes that the anti-inflammatory corticosteroids are actually harmful and therefore contraindicated in numerous infectious lesions. By pre-

venting formation of the chemical factors responsible for inflammation the microorganisms are allowed free rein to proliferate and accentuate the pathological lesions. The anti-inflammatory agents should be employed primarily in non-infectious forms of inflammation. An exception would be in the treatment of infections involving the eye where inflammation leads eventually to corneal opacity.

THE LYMPHOCYTE

This has been called the mystery cell of the body for it has been assigned many functions in hematopoiesis by various authors. It has been claimed that lymphocytes are capable of transformation to granulocytes, monocytes, macrophages, epithelioid cells, giant cells, fibroblasts, and plasma cells.²² Yoffey²⁴ states that lymphocytes constitute 10 per cent of the nucleated cells of the normal marrow and that they serve as stem cells for erythropoiesis and granulopoiesis.

The reason for differences in normal blood levels of lymphocytes among the domestic animals is not known, *e.g.*, mean lymphocyte numbers expressed in per cent of total leukocyte count: dog, 20; cat, 32; cold horse, 35; hot horse, 44; pig, 55; cow, 58; and sheep, 62. Selye¹⁹ in his studies on adaptation observed that the adrenal cortical hormones depress and cause involution of the lymphocytic tissues. It is now an accepted fact that the corticosteroids cause disappearance of lymphocytes from peripheral blood. In animals with a high proportion of lymphocytes such as the mouse, rat, and rabbit,⁷ the injection of ACTH was followed by a decrease in leukocyte count, lymphopenia and neutrophilia. These findings explain why the bovine shows little or no increase in total leukocyte count in the initial response to the stress of infection. The lymphocytes as well as eosinophils are depressed by adrenal cortical activity and if the accompanying neutrophilia is not sufficiently great to compensate for these reductions, the total count actually becomes reduced. There is a parallel between the responsiveness to infection, as expressed in total leukocyte count, and the percentage of lymphocytes common to the blood of the various domestic species in health. Thus, the lower the normal lymphocyte count the greater the responsiveness to stress as reflected in the total leukocyte count.

The lymphocyte and its close relative, the plasma cell, have been assigned a function in the immune response of the host. These cells are said to shed their cytoplasm into the lymph and that the cytoplasm contains gamma globulin and, in the immunized animal, antibody.⁸ Following injection of typhoid antigen or sheep erythrocytes into the foot pad of the rabbit, Harris and colleagues¹¹ were able to demonstrate an antibody titer in the efferent lymph of the popliteal lymph node before it occurred elsewhere in the body. They concluded that the

lymphocytes of the popliteal lymph node were instrumental in the formation of antibodies to the injected antigen. In another trial¹² the popliteal lymph node was removed shortly after injecting antigen into the hind foot pad of the rabbit. The lymph node cells were teased free, washed, sedimented by centrifugation and injected intravenously into a recipient normal rabbit. Antibody to the antigen injected into the donor appeared in the sera of the recipient on the first day after transfer, rose in titer on the second and third day and began to decline on the fifth or seventh day. These results strengthen the supposition that the lymphocytes are involved in antibody formation.

The life span of the lymphocyte is as debatable as the proposed functions of this cell. Life span is held to be a matter of hours, a few days or a month or more. Recent work with P^{32} labeled DNA⁴ indicates a period of 15 days for the time spent in the lymph nodes and blood. This probably represents a minimum life span since undoubtedly the lymphocyte persists for an unknown additional period of time in the tissues after leaving the blood stream.

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Chapter 9

The Leukemia Complex

LEUKEMIA is a neoplastic disease involving one or more of the cell-types of the hematopoietic tissues. The name implies white blood and was first used in 1847 by Virchow to distinguish Weisses Blut (Leukämie) from the leukocytoses of bacterial origin.

Leukemia is a fatal disease and, therefore, a final diagnosis should not be given until the evidence is certain. The problem of leukemia in the human family has received publicity to the point that the animal owner is aware of the fatal outcome of the disease. It can prove to be embarrassing to have stated that an animal has leukemia and to learn later that recovery has taken place.

The diagnosis of leukemia is not always clear-cut for the name is misleading in that the blood leukocyte count is not always elevated and immature neoplastic cells do not regularly appear in the peripheral blood. The Committee on Nomenclature of Cells and Diseases of the Blood and Blood Forming Organs (See Chapter 3) has defined leukemia as a neoplastic disease arising in hemopoietic tissue in which the type cells appear in the blood or are disseminated diffusely through the bone marrow. In characterizing the disease, the type cell should be stated, *e.g.*, lymphocytic, granulocytic or more specifically, neutrophilic, eosinophilic or basophilic, monocytic, or "blast cell," followed by the dual terms leukemic leukemia, subleukemic leukemia, or aleukemic leukemia depending upon the level of blood leukocyte count and presence or absence of the immature neoplastic cells in peripheral blood. In all aleukemic and most subleukemic leukemias bone marrow examination or lymph node biopsy is essential to establish the diagnosis.

COMPARISON OF THE LEUKEMIAS OF MAN AND ANIMALS

In man, the lymphocytic type occurs almost exclusively up to 18 years and beyond 50 years of age, while the granulocytic type predominates between the ages 18 to 50.² The granulocytic type is most common during the procreative period of life when the adrenal gland is dominant; perhaps, the pattern of occurrence of cytologic types of leukemia is influenced by the stimulatory effect of the adrenal cortical hormones on the neutrophilic granulocytes and the simultaneous inhibitory effect of the hormone on lymphocytic tissue. In man leukemias are classified

as "blast cell" (cell type too immature to be identified), which is very acute, granulocytic, lymphocytic, or monocytic. In addition, there is the erythroleukemia or so-called "Di Guglielmo's" syndrome in which the erythrocytic, granulocytic and megakaryocytic elements proliferate *en masse* or as a unit, rather than as a single element.¹⁴ Mast cell or basophilic leukemias also occur in man. In acute leukemia of man, the expected duration of life is 3 months or less from first symptoms. The onset is typically with fever, stomatitis and bleeding into the skin, and from the gums and mucous membranes. In subacute leukemia the expected duration is 3 months to a year. The onset is more insidious but within 3 months from the first symptoms, fever, stomatitis, and bleeding may occur. Chronic leukemia may cover a span of years with periods of remission alternating with relapses.

In animals, the lymphocyte is predominantly involved, irrespective of age of the patient. Terms such as lymphosarcoma, malignant lymphoma, lymphadenosis, and lymphoblastoma are common to veterinary literature dealing with this disease complex. Cell-types other than lymphocytic have been described but appear to be rare in all domestic animals with perhaps the exception of the cat in which a number of cell-types have been described. The disease, generally, is not brought to the attention of the veterinarian in the initial stages, so that the complete course seldom has been studied by the veterinary practitioner and terms reflecting duration such as acute, subacute or chronic, commonly, have not been employed.

Bleeding into the skin or from the gums and mucous membranes, as a consequence of associated thrombocytopenia, is not a characteristic feature of the leukemias in animals, although Holzworth⁴¹ has stated that in the cat, "hemorrhagic tendencies develop with liver failure, and also because of depressed platelet formation when the marrow is involved." The leukemia complex is typically afebrile in the dog and cow, while the cat differs again in that rectal temperatures of 104–105° F. are commonly recorded. In man, the leukemia complex is associated with an anemia, often of severe degree. In animals, severe anemia is characteristic of the disease in the cat, a moderate anemia can be anticipated in the dog, whereas in the cow, the occurrence of anemia is variable and not necessarily a characteristic feature of the leukemia complex.

Hodgkin's disease in man consists of a granulomatous process in the lymph nodes which begins with lymphocytic hyperplasia followed by a gradual loss of normal architecture with replacement of the lymphocytes as the disease progresses. Mononuclear and multinuclear giant cells, called Reed-Sternberg cells, are pathognomonic and their presence is essential for a diagnosis. Eosinophils are usually numerous in the lymph nodes. The organs involved in descending order are lymph nodes, spleen, liver, bones (leading to anemia) lungs and the miscella-

neous organs. The etiology has not been established with absolute certainty but it appears to be viral in origin. Typical Hodgkin's disease has not been shown to occur in animals, although Moulton and Bostick³⁹ have reported on a case of malignant lymphoma in the canine simulating the disease. Meier³⁸ lists 6 possible cases of Hodgkin's sarcoma in the dog based on the occurrence of pleomorphism and giant cells of the Reed-Sternberg type.

It would be well to state at this juncture that the condition known as infectious mononucleosis in children and young adults apparently has no counterpart in animals.

FACTS AND THEORIES REGARDING THE ETIOLOGY OF LEUKEMIA

Transmissible Agent. A transmissible, filterable agent was reported as a cause of leukemia in chickens in 1908 by Ellermann and Bang.¹ Mammalian leukemia has been investigated almost exclusively in certain inbred strains of mice which show a high incidence of spontaneous lymphocytic leukemia. In 1951, Gross⁶ reported that filtered AK mouse leukemic tissue extracts inoculated into infant mice of the C3H (f) strain, when less than 12 hours old, resulted in development of leukemia at 6½ to 9 months of age in 7 (28 per cent) of 25 mice exposed. Such inoculated mice were mated upon reaching sexual maturity and leukemia developed in 13 (48 per cent) of 27 offspring at 14 to 20 months of age. The incidence of spontaneous leukemia in untreated mice of the C3H (f) colonies was reported to be only 0.07 per cent. Thus, a transmissible filterable agent was demonstrated for the first time in mammalian leukemia and an additional significant finding was that the filterable agent was transmitted from parent to offspring. The leukemic agent proved to be highly specific, requiring particular strains of mice for successful transmission.¹ Extensive verifications of these observations have been made by the original investigators and others.

Genetic Influence. Experimental studies on the transmission of leukemia have clearly demonstrated that inbred lines of chickens and mice give more uniform results than heterogeneous animals. Richter and MacDowell¹² developed a mouse strain in 1921 from brother-sister matings which they designated strain C58. A high incidence of lymphocytic leukemia develops in mice of this strain living beyond 8 months of age. All mice of the C58 strain were found susceptible to experimental leukemia when inoculated with mouse leukemic cells. On the other hand, mice of the Storrs-Little strain were highly resistant to leukemia when inoculated with the same cell lines employed on C58 mice. By cross breeding the two strains and back-crossing and testing the progeny for susceptibility to leukemia, Richter and

McDowell¹³ concluded that both genetic and non-genetic factors play a part in development of leukemia and that in the case of the C58 strain, the genetic factors are 9 times as strong as non-genetic factors. Furth, Rathbone and Seibold⁵ studied 5 transmissible cell strains of mouse lymphomatosis and state that 2 strains could be transmitted to any stock of mice but 3 strains to related mice only. Kirschbaum and Kaplan⁷ summarized their observations as follows: "The problem of leukemogenesis in mice is very complex—first, multiple agents can induce leukemia; second, mice of only certain genetic constitution are susceptible to only certain agents; third, genetic susceptibility to one agent, or to the spontaneous disease, cannot necessarily be correlated with susceptibility to other leukemogenic agents."

Ionizing Radiation. Furth³ was the first to make the observation that leukemia may be induced by exposure to x-rays. Myeloid leukemia was rarely found in the mouse colony (low-leukemia index) until a large number of mice had been exposed to a single dose of 400r of x-rays. Furth and co-workers⁵ also observed that a single exposure to x-rays increased the susceptibility of mice to transmissible lymphomatosis. The success of inoculations increased with x-ray dose and was almost 100 per cent when the mice received 400 to 600r. The duration of susceptibility to the inoculated leukemic cells was proportional to the quantity of irradiation. At a dose level of 400–600r, about one-third of the mice remained susceptible to leukemic-cell inoculations for 2 to 3 months, but more than one-half of them regained resistance after 1 to 2 months.

The carcinogenic hydrocarbon methylcholanthrene was reported to induce lymphomatosis and leukemia in 20 per cent of mice of low-leukemia index when painted on the skin.¹¹ The chemical was also found to cause leukemia to appear at an earlier age when applied to the skin of high-leukemia strains of mice.⁸ Pre-irradiation was found to greatly enhance the susceptibility of mice to the leukemogenic action of methylcholanthrene and pre-irradiation also hastened the onset of leukemia.⁴

The exposure of humans to radiation energy either accidentally or in the course of medical treatment either as patient or radiologist has led to a number of papers showing a correlation between such exposures and the subsequent development of leukemia. Perhaps, the most convincing is the report by Moloney and Kastenbaum¹⁰ on the leukemogenic effects of ionizing radiation on atomic bomb survivors in Hiroshima. Among the 98,000 survivors there were 50 cases of verified leukemia up to 1955; the incidence was distributed in a fashion that revealed a parallel between distance from the epicenter and frequency of development of leukemia.

A case of lymphocytic leukemia has been described as appearing in

one of 56 hogs given 50r daily of gamma radiation from Cobalt-60. The leukemic hog was the thirty-sixth of the group to die (196th day of exposure) but all the others died of chronic irradiation sickness.¹⁵

March⁹ has made a statistical analysis of the frequency of occurrence of leukemia among medical personnel. The study is reported to have revealed that leukemia occurs 9 times more frequently in radiologists as in non-radiologist physicians.

Occurrence of Leukemia Among Survivors of Hiroshima Atomic Blast

<i>Distance in Meters from Epicenter of the Blast</i>	<i>Population (Survivors)</i>	<i>Cases of Leukemia</i>	<i>Incidence</i>
0-999	1,200	15	1:80
1,000-1,499	10,500	24	1:438
1,500-1,999	18,700	5	1:3,740
2,000-2,499	17,200	2	1:8,600
2,500-over	50,500	4	1:12,625

It is not possible at present to relate the findings of research on mouse leukemia to leukemia of the larger domestic mammals. In general, cases of leukemia in animals have been of a sporadic nature and such material does not lend itself to the elucidation of cause and effect. However, in cattle, herds having a high-leukemia incidence occur in Northern Germany and Scandinavia. Recently, such a herd has been encountered in California and studies of the nature of the disease both in California and the European countries are underway.

THE LEUKEMIA COMPLEX IN THE BOVINE

In the bovine, the recorded cases of neoplasia originating in the hematopoietic tissues have been almost exclusively lymphocytic. Recently a case of granulocytic leukemia was described in a 4-month-old female Angus-Holstein crossbred²⁴ which was presented for necropsy after having died on the 9th day of illness. A differential leukocyte count in blood revealed the following percentages of cells: blast cells, 27; progranulocytes, 13; myelocytes, 27; metamyelocytes, 3; band neutrophils, 1; segmented neutrophils, 1; lymphocytes, 15; and unclassified cells, 13. Bone marrow impressions showed 70 per cent of the cells to be infiltrative blast-types. The authors state that a review of the literature did not reveal a single report of a well-substantiated case of granulocytic leukemia in the bovine.

Hatzios^{23a} described a lymphoblastic lymphoma in a stillborn fetus expelled by a normal primiparous Shorthorn cow, at the eighth month of pregnancy. This author stated that only 2 cases of malignant lymphoma have been recorded in bovine fetuses, namely, one at 113 days and

another at 210 days of intrauterine life, and these from the German literature. Bendixen¹⁷ of Denmark has made reference to 8 cases of "leucosis" found among newborn calves and young animals up to 1½ years of age. The latter author concluded that the cases in calves were unrelated to the herd problems of leukemia under study in Denmark.

A sporadic form of lymphocytic leukemia in the bovine has been reported frequently in the American Veterinary literature as lymphosarcoma,^{19,28,29,31} lymphoblastoma,^{18,21,26} lymphocytoma,^{25,30,} and leukemia.²⁷ Because of the very marked and extensive neoplastic involvement of lymphocytic foci throughout the body and the infiltration of malignant cells into almost every organ, the terms lymphosarcoma or lymphocytoma are more descriptive of the over-all nature of the process than the term leukemia.

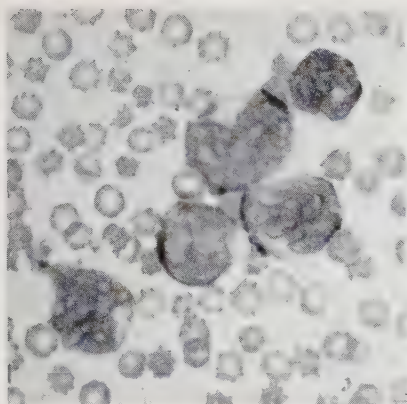
Clinical Signs and Differential Diagnosis. The leukemia complex may affect cattle of all ages, but it is more commonly encountered in animals 5 years of age and older. It is characteristically afebrile and may appear to develop suddenly with rapid wasting and death; however, this is only the terminal stage, for the disease process may have gone unnoticed over a period of months. In 1 cow, bilateral enlargement of the prefemoral lymph nodes was followed by remission and a year later a rapid terminal phase developed²⁸ (see Table 38).

Because of the marked involvement of such a variety of tissues and organs the clinical signs may be quite variable. Bilateral enlargement of superficial nodes may be the first detectable abnormality, although in some instances only a single external node is involved and in other cases no enlargement of external lymph nodes occurs. When lymphosarcoma is suspected, rectal palpation of the pelvic and abdominal organs and tissues may reveal extensive neoplastic masses.

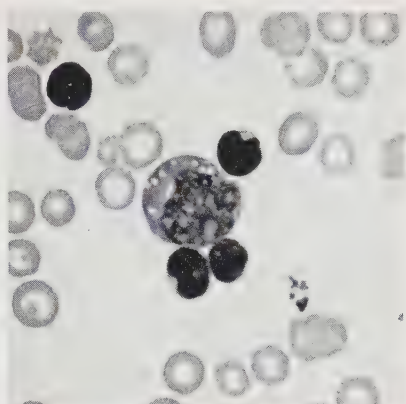
The abomasum is commonly infiltrated¹⁹ and clinical signs of chronic indigestion may appear. The myocardium is also a favored site for infiltration and when chronic indigestion and cardiac abnormality co-exist, the clinical signs may suggest a case of traumatic pericarditis. The pressure of neoplastic masses on nerves or the spinal cord may produce partial or complete paralysis depending on the location of the lesion.^{30,31} In rare cases, the subcutaneous tissues and skin may be involved with a considerable number of nodules of varying size that need to be distinguished as to their true nature. Infiltration of neoplastic lymphocytes into the mammary gland has been reported,¹⁸ and Theilen observed a case of lymphosarcoma in which neoplastic cells were present in the milk and this was associated with a positive California mastitis test reaction.

The Blood Picture. In addition to the total and differential leukocytes counts, it is important to note the presence of immature lymphocytes or cells of atypical morphology.

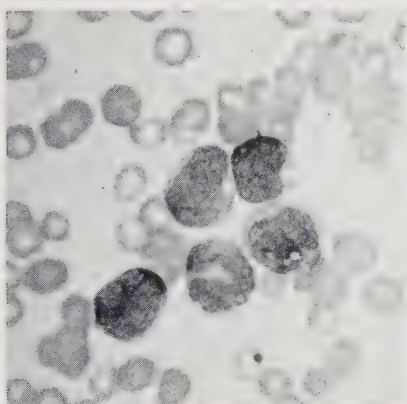
PLATE X. THE LEUKEMIA COMPLEX. 600 ×



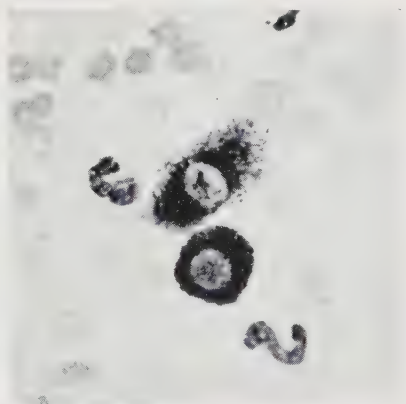
Bovine lymphocytic leukemia. Lymphoblasts and prolymphocytes.



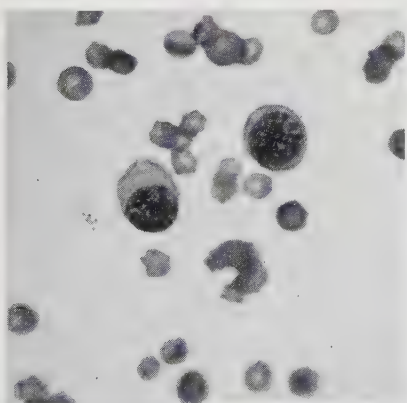
Canine lymphocytic leukemia. A large lymphoblast and four small lymphocytes.



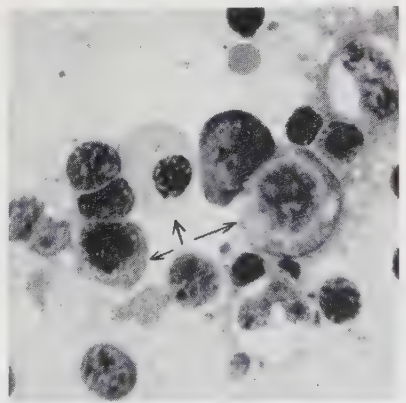
Canine monocytic leukemia. Five cells showing monocytoïd characteristics.



Feline basophilic leukemia. Extreme basophilic granulation was characteristic.



Feline erythroleukemia (blood). Two abnormal giant rubricytes.



Feline erythroleukemia (bone marrow). Arrows: abnormal erythrocytic cells.

Thompson *et al.*^{21,30} stated that chief reliance should be placed on the number and type of lymphocytes and they concluded that in most cases a marked change in lymphocyte morphology can be detected. Feldman²⁰ stated that less than 50 per cent of lymphosarcoma cases reveal excessive numbers of lymphocytes in the blood stream.

Table 38. Hemograms of a Case of Lymphosarcoma in a 7-year-old Holstein Cow Kept under Observation for 13 Months During Which Time a Period of Remission Occurred in the Clinical Picture

Date	PCV	WBC	Band	Neut.	Blast	Pro- lym- pho- cyte	Atyp- ical Lym- pho- cyte	Lym- pho- cyte	Mono.	Eos.	Bas.	D.C.
Nov. 18	26	9,850	0	55.0	0	14.0	0	14.0	15.0	2.0	0	0
Dec. 2	28	10,800	0	4.5	0	5.0	7.5	57.0	10.5	13.5	0	2.0
Jan. 7	31	22,000	0	18.5	0	5.0	0	62.0	4.0	10.5	0	0
Mar. 18	30.5	11,850	0	38.0	1.0	1.0	0	47.0	2.0	10.0	1.0	0
Apr. 29	32	9,400	0	27.0	0	0	4.0	63.5	0.5	5.0	0	0
June 12	30	12,400	0	27.0	0	0	3.0	64.0	1.5	4.0	0.5	0
July 15	36	8,600	0	31.0	0	0	2.0	59.0	5.0	2.5	0	0.5
Aug. 12	35	10,850	0	35.5	0	0	1.5	57.0	3.5	2.5	0	0
Oct. 6	36	12,200	0	30.5	0	0	2.0	54.0	8.0	5.5	0	0
Nov. 25	33	28,050	0.5	9.0	0	0	56.0	31.0	1.5	2.0	0	0
Dec. 10	21.5	95,000	0.5	2.0	0	0	85.5	11.5	0	0.5	0	0
Dec. 14	28	143,000	0	4.0	0	0	94.0	2.0	0	0	0	0
Dec. 18	23	142,000	0	3.0	0	0	88.0	9.0	0	0	0	0

Necropsy revealed the following to be involved: all lymph nodes, tongue, abomasum, spleen, myocardium, uterus, ureters, lungs and kidneys.

Theilen, Schalm and Gilmore²⁹ in studying a herd problem of lymphosarcoma divided the lymphocytic series into lymphoblast, prolymphocyte, and lymphocyte. The lymphoblast generally is a large cell with at least one nucleolus which stains light blue in contrast to the deeper purple of the rest of the nucleus, and generally there is more cytoplasm in relation to the nucleus than is common with the large, mature lymphocyte. The prolymphocyte is a large cell with considerably more cytoplasm than is common to mature lymphocytes, and the nucleus generally presents one or more ring-like structures representing nucleolar remnants (Plate X). Generally the cytoplasm of immature or neoplastic lympho-

cytes is more basophilic and the nuclear chromatin is more diffuse than with the mature lymphocyte. Binucleate cells and mitotic figures, as well as cells that are neither typically lymphoblasts, prolymphocytes, or mature lymphocytes may be encountered and their numbers may vary from time to time in the same animal (Table 38).

The total leukocyte count may vary from frank leukopenia through the normal range to marked leukocytosis (Table 39). In the case

Table 39. Examples of Hemograms in 10 Cases of Bovine Lymphosarcoma

Case No.	Age	Sex	PCV	WBC	Band	Pro-						
						Neut.	lymph.	Lymph.	Mono.	Eos.	Bas.	D.C.
53L40	5 wks.	F	12	2,050	18.0	2.0	28.0	50.0	2.0	0	0	0
57L1672	8 mo.	F	34.5	8,400	0	15.0	0	80.0	4.0	1.0	0	0
56L15	2½ yr.	M	42	5,250	0	21.5	0	59.5	5.5	13.0	0.5	0
56L937	3 yr.	F	28.5	6,800	0	47.5	0	33.0	14.0	5.5	0	0
961A	4 yr.	F	28	175,000	0	6.0	83.0	6.0	0	0	0	5.0
53L114	5 yr.	F	28	12,450	0	72.0	0	15.0	11.0	1.0	0	1.0
57L1400	Mature	F	34	20,700	0	51.5	0	42.0	6.5	0	0	0
RJO-68	Mature	F	34	19,100	0.5	3.0	16.0	79.5	0	1.0	0	0
RJO:329	Mature	F	35	145,000	0	2.0	57.0	39.0	0	0	0	2.0
UCV-65	Mature	F	25.5	198,750	0	14.0	2.5	38.5	45.0	0	0	0

presented in Table 38 an extremely high terminal peripheral leukocyte count was observed. Several additional cases exhibiting marked leukemia at termination appear among the cases detailed in Table 39. These findings are in contrast to the observations of Frank and Thompson²¹ in Kansas where marked leukocytosis initially was followed by a steady decline in peripheral leukocyte count with leukopenia developing before death. Several of the cases in Table 39 could not be diagnosed from the blood leukocyte picture.

Anemia may be associated with lymphocytic leukemia in the bovine but it is of variable occurrence and it is not a characteristic feature of the disease.

Lymphosarcoma Herds and Their Detection. The incidence of lymphosarcoma in the bovine in the United States, based on federal meat inspection reports, was about 1.7 cases per 10,000 head slaughtered in 1958. Viewing the reports from 1945–1958, the number of cases of lymphosarcoma being encountered had doubled.²⁹ An increased fre-

quency of the disease²¹ was noted in Kansas after 1935. As yet it cannot be stated whether or not the change in frequency is merely apparent due to more careful reporting or real. In any event, the observations coming out of Denmark¹⁶ and Germany²² pointing to herd problems and the possibility of the contagious nature of the disease should direct attention to the entire herd whenever a case of lymphosarcoma is diagnosed in the bovine.

Table 40. Goetze's Key for Classifying Bovines Relative to Lymphosarcoma Based on Age, Total Leukocyte Count, and Per Cent Lymphocytes

<i>Total Leukocytes Counts Based on Age</i>		<i>Per Cent Lymphocytes</i>		
<i>Up to 2 Years</i>	<i>3+ Years</i>	<i>To 65</i>	<i>65-75</i>	<i>75+</i>
to 12,000	to 10,000	Negative	Negative	Suspect
12,000-18,000	10,000-18,000	Negative	Suspect	Positive
18,000+	18,000+	Suspect	Positive	Positive

Table 41. Bendixen's Key for Classifying Cows Relative to Lymphosarcoma Based on Age and Absolute Lymphocyte Count

<i>Age in Years</i>	<i>Normal</i>	<i>Suspect</i>	<i>Positive</i>
0-1	< 10,000	10,000-12,000	> 12,000
1-2	< 9,000	9,000-11,000	> 11,000
2-3	< 7,500	7,500- 9,500	> 9,500
3-4	< 6,500	6,500- 8,500	> 8,500
4	< 5,000	5,000- 7,000	> 7,000

A herd study requires blood samples to be drawn from all mature cattle for the total and differential leukocyte counts. Goetz and co-workers²³ in Germany classify the individual animal as normal, suspect, or positive for lymphosarcoma by combining the total leukocyte count and the percentage lymphocytes (Table 40).

Bendixen¹⁷ in Denmark has developed a lymphosarcoma classification key that employs the absolute count of lymphocytes adjusted to age of the animal (Table 41).

Theilen, Schalm, and Gilmore²⁹ have investigated the lymphosarcoma problem in a purebred Milking Shorthorn herd in which clinical lymphosarcoma was diagnosed 5 times and twice confirmed histopathologically over a 5-year period. Two complete clinical and hematologic investi-

gations on 50 cows were conducted with 14 months intervening. The cows were classified as positive for lymphosarcoma on the basis of absolute lymphocyte counts above 9,000 or if lymphoblasts and/or 4 per cent or more prolymphocytes were present irrespective of the level of total lymphocytes. Normal leukocyte and lymphocyte values were established from data obtained within the herd to show the influence of aging on circulating leukocyte number and absolute lymphocytes. The data from this single herd are summarized in Table 42.

Table 42. Ranges and Means for Total Leukocytes and Lymphocytes of Cows of Various Ages in a Single Purebred Dairy Herd and Their Classification as Normal or Positive with Respect to Lymphosarcoma*

Class	Age in Years	Number of Cows	Total Leukocyte Count/cmm.	Blast Cells %	Pro- lymph. %	Lymph. %	Total %	Absolute Total Lymphocytes/cmm.
Normal	2-5	15	5,400-12,300 (9,093)	0	0-3	34-79	59	2,924-8,241 (5,353)
	5-9	5	4,850-7,600 (6,440)	0	0-2	36-63	46	1,794-3,382 (2,922)
	10-17	7	5,250-7,900 (6,060)	0	0-2	37-59	47	1,804-4,661 (2,874)
Positive	2-5	5	12,950-22,050 (16,525)	0-2	1-30	46-85	75	9,782-17,775 (12,539)
	5-9	5	11,000-20,250 (16,910)	0-2	1-22	67-92	81	9,728-18,225 (14,356)
	10-17	7	11,550-50,200 (21,146)	0-5	0-72	17-86	79	7,456-47,188 (16,917)

* Six cows were classified as lymphosarcoma suspects and are not included in this table.

The normal cows showed a reduction of total leukocyte count by one-third and of the absolute lymphocyte count by one-half after 5 years of age. On the other hand, cows classified as positive for lymphosarcoma had total leukocyte counts above 11,000 per cmm., regardless of age. Due to the significant reduction in total circulating lymphocytes after 5 years of age in normal bovines, it is reasonable to consider that a number of lymphocytes less than 9,000 per cmm. may be indicative of lymphosarcoma in older cows. Bendixen in his key (Table 41) uses the number 7,000 as indicative of a lymphocytic leukemic process in cows 4 years of age or older.

A definite increasing-with-age relationship of blood findings positive for lymphosarcoma was shown, *i.e.*, 22.6 per cent of cows 2-5 years old, 38.4 per cent of cows 5-9 years old, and 46.6 per cent of cows 10-17 years old. Despite this high incidence of positive cows, by hematologic

standards, clinical lymphosarcoma was not at the time a prominent feature. Goetze²² comments that cows may remain in a preclinical stage with positive blood findings for months and even years, but this situation may change at any time into a fatal tumorous form in a low percentage of the leukemic animals.

LEUKEMIA COMPLEX IN THE CANINE

Bloom and Meyer,³³ Innes, Parry and Berger,³⁵ and Jennings³⁶ have stated that the leukemia complex in the dog is probably limited to the lymphocytic type. Meier³⁸ has recorded 8 cases of granulocytic leukemia and 1 case of monocytic leukemia as compared to 52 cases of lymphocytic origin. The incidence of the leukemia complex in dogs admitted to veterinary clinics was reported by Bloom³³ to be 1 in 500 and by Meier³⁸ as 1 in 1,000 cases.

Bloom and Meyer³³ suggested malignant lymphoma as a more appropriate name for neoplasia of lymphocytic origin in the canine by reason of the fact that the peripheral blood picture is more often not diagnostic, and in some cases there appears to be a non-specific leukocytosis involving the neutrophilic series. Innes *et al.*,³⁵ state that aleukemic and leukemic cases are seen and in some instances, with typical gross pathology, the blood picture resembles a non-specific, neutrophilic leukocytosis. Jennings³⁶ in summarizing 8 cases stated that, while all cases were well-defined clinically, most of the dogs presented normal hemograms. On this same point, Meier concluded that the blood findings are inconsistent, seldom leukemic and either normal or characteristic of neutrophilic leukocytosis. A summary of 16 cases is presented in Table 43. Two of these cases were extremely leukemic with leukocytes exceeding 500,000 per cmm., with the majority of cells being of lymphocytic origin (Plate X). Five additional cases were verifiable on the basis of occurrence of lymphoblasts, prolymphocytes and/or a significantly high percentage of mature lymphocytes. Two cases were representative of a modest neutrophilic leukocytosis associated with a relative lymphopenia in which the absolute lymphocyte number was at the lower end of the normal range.

Clinical Signs of Malignant Lymphoma. The disease is most commonly observed after 5 years of age and apparently affects both sexes equally. The onset is insidious and little information is at hand relative to duration. Bloom³³ reports that in 5 cases where complete histories, at least during the period of illness, were available, deaths occurred in 39 to 163 days after first symptoms and averaged 99 days. One of our cases studied in detail and summarized in Table 44 was under observation for 18 months. This case was unique in that there was a complete absence of enlargement of external lymph nodes, but there existed an

Table 43. Clinical Data and Hemograms in Malignant Lymphoma in the Canine

Case No.	Age	Sex	Temp.	Lymphadenopathy	PCT	MCV	Sed. Rate	Nuc. RBC	WBC $\times 10^3$	Meta.	Band	Neut.	Blast	Pro-lymph.	Lymph.	Mono.	Eos.	D.C.
54S106	5	M	100.8	(Bone marrow +) All enlarged	20	66	6/-43	1.0	18.4	0	3.0	36.0	3.0	14.0*	12.0	2.0	1.0	28.0
54S272	12	F	102.0	(Bone marrow +) All enlarged	23	58	13/-27	8.0	16.8	0	2.0	48.0	3.0	8.0*	12.0	1.0	0	26.0
54S1213	8	F	101.4	Cervical and Popliteal	46	60	39/+35	0	26.4	0	8.0	71.0†	0	0	3.0	17.0	0	0
55S2163	7	F	103.2	All enlarged	38	60	5/-7	0	8.7	0	0.5	58.0	0	0	19.0	21.0	0.5	0
55S2708	6	F	101.4	Extreme	41	77	0/-9	0	9.6	0	1.0	81.0	0	0	5.0	10.0	2.5	0.5
55S2780	7	M	101.2	(Bone marrow +) Tonsils	34	75	8/-10	0	19.2	3	4.0	68.5	1.0	18.5*	3.5	0	0	1.5
56S460	4	F	102.5	Many enlarged	48	71	4/+2	0	9.8	0	0	68.0	0	1.0	23.0	8.0	0	0

55S350	13	?	100.5	Large mass left costal area	31	55	1/-23	0	23.0	0	3.0	88.0†	0	0	3.5	5.0	0.5	0
Ac4938	3	M	?	All enlarged	21	72	70/+24	0	575.0*	0	0	2.0	0	2.0	95.0	1.0	0	0
56S1797	8	M	101.0	None external Few internal	25	77	2/-34	2.0	524.0*	0	0	0.6	0.4	24.0	68.0	0.4	0	6.6
55S3530	5	F	101.8	Some nodes involved	32	70	5/-17	0.5	30.7	0	2.0	26.0	0	6.0*	60.0	3.0	3.0	0
57S2201	6	M	102.1	All enlarged	43	73	12/+5	0	11.7	0	0	73.0	0	0	17.5	8.5	1.0	0
58S1798	8	F	102.5	All enlarged	45	67	16/+11	0	18.0	0	0	83.0	0	0	12.5	3.0	1.5	0
58S1976	3½	M	99.7	All enlarged	52	71	0/0	0	8.3	0	1.3	55.7	0	0	32.0	6.0	5.0	0
59S257	6½	M	103.5	All enlarged	38	82	5/-7	0	19.4	0	0	89.5	2.0*	0	4.0	3.0	1.5	0
56S287	10	M	100.9	Many enlarged	48	68	0/-2	1.0	7.7	0	1.5	68.0	0	0	22.0	6.0	2.0	0

* Positive peripheral blood for the lymphocytic leukemia complex.

† Non-specific neutrophilic leukocytosis.

Table 44. *A Case of Lymphocytic Leukemic Leukemia in the Canine*

A boxer male, 8 years old, was presented with the complaint of vomiting and loss of weight. The temperature was 101.8 and throughout the period of observation the temperature remained within the normal range. The case was unusual in that there was no lymphadenopathy or swelling in the abdominal cavity. The patient maintained his weight for about 15 months and then over the final 3 months, a rapid and marked weight loss took place and this coincided with the fall in the total leukocyte count. Patient was euthanized because of extreme cachexia.

Date	PCV	MCV	MCHC	Sedimentation Rate	Retic. (1)	WBC	Band	Neut.	Blast	Pro-lymph.	Lymph.	Mono.	Eos.	D. C.
Sept. 21/56	25.5	67.0	33	1/-34	0.4	348,000	0.0	1.0	0.0	3.0	87.0	0.5	0.0	8.5
Sept. 26	24.0	68.5	32	6/-32	0.0	418,000	0.0	0.8	0.0	9.2	90.0	0.0	0.0	0.0
Oct. 2	25.0	77.0	31	2/-34	0.0	524,000	0.0	0.6	0.4	24.0	68.0	0.4	0.0	6.6
Oct. 5	22.5	72.0	33	*20/-21	0.1	403,000	0.0	2.0	0.0	7.0	90.0	0.0	0.0	1.0
Oct. 9	24.0	70.0	32	*30/-8	0.0	580,000	0.0	0.2	0.0	6.8	89.6	0.2	0.0	2.6
Oct. 29	22.5	74.0	33	*35/-6	0.5	540,000	0.0	1.6	1.0	7.0	89.6	0.2	0.0	0.6
Nov. 7	22.5	68.0	33	*16/-25	0.5	498,000	0.0	2.2	0.2	3.2	93.5	0.2	0.0	0.8
Nov. 7	Bone marrow aspiration, 2,000 cells differentiated—86.0% mature lymphocytes 4.6% polymorphocytes. Only 186 cells were of the granulocytic and erythrocytic series. The Myeloid: Erythroid ratio base on these 186 cells was 2.2.													
Dec. 14	24.5	76.0	32	*15/-22	0.1	482,000	0.0	1.0	0.0	2.6	96.2	0.2	0.0	0.0
Jan. 24/57	24.5	63.0	32	27/-10	1.2	544,000	0.0	1.4	0.2	2.6	92.0	0.0	0.0	4.0
Feb. 19	25.0	75.0	33	15/-21	0.7	342,000	0.0	0.2	0.0	5.5	94.3	0.0	0.0	0.0
Mar. 19	23.0	65.0	33	*51/+11	0.3	276,000	0.0	2.5	0.0	0.0	95.5	2.0	0.0	0.0

May 1	20.0	60.0	31	*70/+21	0.1	393,000	0.5	3.0	1.5	6.0	83.0	6.0	0.0	0.0
July 1	19.0	71.0	36	56/+4	0.1	106,000	0.0	2.0	0.0	0.0	97.0	1.0	0.0	0.0
Sept. 4	24.0	63.5	32	41/+3	0.8	360,000	0.0	3.3	0.0	0.0	95.3	0.7	0.7	many
Oct. 3	28.0	61.0	31	47/+17	1.1	277,000	0.2	3.2	0.0	0.0	92.8	0.0	0.6	0.0
Oct. 18	25.0	79.0	31	62/+26	0.6	172,000	0.6	2.2	0.2	3.6	92.6	0.6	0.2	0.0
Nov. 1	24.0	63.0	32	59/+21	2.0	197,000	0.0	8.0	0.0	0.0	90.0	1.5	0.5	0.0
Nov. 15	27.5	78.0	32	27/-4	1.8	136,000	1.6	4.6	0.0	0.0	93.8	0.0	0.0	0.0
Nov. 22	26.5	83.0	33	*20/-13	2.3	99,500	0.0	5.0	0.0	4.5	87.0	2.0	0.0	1.5
Dec. 6	26.0	78.0	31	*27/-7	0.2	56,000	1.5	6.0	1.5	5.0	73.5	12.5	0.0	0.0
Dec. 13	24.0	78.0	31	*35/-3	1.8	48,000	0.5	2.5	0.5	5.5	90.0	0.0	0.0	1.0
Dec. 20	24.5	74.0	31	*20/-17	1.3	29,300	0.5	9.5	2.0	1.5	86.0	0.5	0.0	0.0
Dec. 27	29.5	67.0	30	*24/-3	5.5	18,400	0.0	0.5	0.0	1.5	87.0	2.0	0.0	0.0
Jan. 3/58	25.0	76.0	29	*25/-11	3.0	11,500	3.5	13.0	0.0	6.0	74.0	3.0	0.0	0.5
Jan. 10	20.5	86.0	29	*30/-18	0.2	10,600	2.0	21.5	0.0	5.5	66.0	2.0	0.5	2.5
Jan. 17	26.0	86.0	30	*17/-17	2.4	8,150	1.5	21.0	2.0	5.0	66.0	3.0	0.0	1.5
Jan. 24	24.0	87.0	29	*20/-18	1.9	6,600	5.0	23.0	1.0	3.0	62.0	6.0	0.0	0.0
Jan. 31	25.0	71.0	29	*25/-11	—	8,000	0.0	39.0	0.0	3.0	51.0	7.0	0.0	0.0
Feb. 10	22.0	75.0	31	27/-16	1.6	4,700	0.0	31.0	0.0	0.0	59.0	10.0	0.0	0.0
Mar. 3	26.0	83.5	31	*12/-22	2.2	8,500	0.0	44.0	0.0	2.0	45.0	9.0	0.0	0.0
Mar. 10	24.0	81.5	30	*19/-19	7.7	6,300	0.0	38.0	0.0	1.0	57.5	3.5	0.0	0.0
Mar. 25	24.0	87.0	31	30/-8	0.8	12,100	3.0	64.5	0.5	6.0	28.5	4.0	0.0	0.0

* Diphasic erythrocyte sedimentation rate. (1) from 1 to 4 nucleated red cells/100 WBC were commonly present with a high of 10.5/100 WBC on January 3, 1958.

Necropsy report. Spleen enlarged 2-3 fold and paler than normal. Lymph nodes = mediastinals not enlarged, mesenterics darkened with $\frac{1}{2}$ mm. white bumps (scotchgrain); post-cervical node creamy white and very juicy; anterior cervical 3" long; lumbar, renal and hepatic nodes markedly enlarged. Differs from usual form of lymphosarcoma by absence of diffuse involvement of nodes. Even the spleen enlargement was caused mostly by proliferation of RE cells.

extreme lymphocytic leukemia. The peripheral leukocyte count declined gradually over the last 5 months and in the last 3 months, it was within the normal range although the lymphocyte percentage remained above normal. The patient appeared to have reached an equilibrium with the disease during the first 15 months of observation but as the leukocyte count fell, the patient's condition deteriorated with rapid and marked weight loss. Due to the complete absence of lymphadenopathy of external nodes or tonsils, this case would have been difficult to diagnose without blood studies.

The first clinical signs in canine malignant lymphoma may be bilateral painless enlargements of lymph nodes or in some instances the first complaint is enlarged tonsils. General health does not appear to be affected at first, but in time listlessness, inappetence, vomiting, diarrhea, dyspnea, choking and coughing, and edema of the face, throat and/or limbs may make their appearance. Some of these signs are definitely referable to mechanical pressure and obstruction resulting from the lymphadenopathy. Listlessness and lethargy may find a partial explanation in the moderate anemia that is sometimes associated with the disease. The edema may be more pronounced at one time than another. Edema usually is not seen until late in the disease. Terminally, there is complete anorexia and rapid weight loss. A helpful clinical finding in differential diagnosis is the generally afebrile nature of the disease.

A clinical disease suggestive of malignant lymphoma should have a careful blood examination with particular attention given to the morphology of the lymphocytes. In a few instances, the leukemic nature of the blood will verify the diagnosis, but in the absence of a leukemic blood and immature or atypical lymphocytes in peripheral blood, a lymph node biopsy for histopathologic examination and/or a bone marrow aspiration can be most helpful.

Granulocytic Leukemia. This form of the leukemia complex is commonly referred to in the literature as myelogenous leukemia. However, in keeping with the recommendations of the Committee on Nomenclature (Chapter 3), the terminology granulocytic leukemia will be adhered to. Meier³⁸ reported observing 8 cases among 100,000 dogs examined at the Angell Memorial Hospital, Boston, between the years 1946-56. According to Meier, the principle gross lesions consisted of an increased amount of bone marrow and spleno-hepatomegaly. Microscopically there was an enormous increase in the number of circulating leukocytes in all developmental stages from undifferentiated non-granular forms to polymorphonuclear mature cells. There was hyperplasia of the bone marrow and myeloid metaplasia of the spleen, liver, lymph nodes and other organs. Severe myelophthisis resulted in marked anemia from interference with erythropoiesis.

Meier³⁸ presented blood leukocyte counts for 6 of his 8 cases and

while 1 case had a normal count of 16,300, the other 5 cases had leukemic blood as follows: 80,750; 186,000; 250,000; 300,000; and 467,000. It would appear that high peripheral blood leukocyte counts may be characteristic of granulocytic leukemia. Clinical signs shown by the 8 cases in order of frequency of observation were as follows: poor appetite or anorexia, progressive weight loss, vomiting, polydipsia and polyuria, lethargy or depression, weakness, pallor, dyspnea, facial edema or edema of other parts of the body, and diarrhea. Rectal temperatures were generally within the normal range but with 1 case the temperature was elevated to 105° F.

Roscher *et al.*^{40a} observed a case of acute granulocytic leukemia in a 2-year-old Boxer. The blood leukocyte count was low on admission (5,000/cmm.) but gradually increased to 30,000/cmm. of blood over a period of 20 days and then fell again to a level of leukopenia in the week prior to death. By use of the peroxidase staining method and a special method for demonstration of alkaline phosphatase in leukocytes, the authors concluded that the neoplastic cells in blood and bone marrow were indisputably of granulocytic origin. Electrophoretic pattern of plasma proteins revealed a marked increase of gamma globulin, whereas other protein fractions remained essentially normal. The plasma coagulation factors, with the exception of fibrinogen, compared favorably with those of normal dogs. Fibrinogen, expressed in mg. per cent, was 31.0 as compared to 407 and 580 in the plasma of normal dogs. At necropsy there was evidence of marked recent weight loss. There was a scant amount of subcutaneous fat and the body cavities were free of significant amounts of fluid. The axillary, cervical, inguinal, and mesenteric lymph nodes were enlarged, the largest being 2.5 cm. in diameter. The nodes were soft and pale gray on section. The histopathology of the liver, spleen and lymph nodes was said to be typical of the cellular pattern characteristic of granulocytic leukemia.

Monocytic Leukemia. Meier³⁸ records 1 case and refers to 1 literature report. A 4-year-old, shepherd-type female dog was presented to our clinic showing marked depression, a chronic cough, weakness of the hind quarters and a temperature of 105° F. Other findings on initial examination were congested conjunctiva, injected sclera, some ocular discharge, muco-purulent nasal discharge and enlarged tonsils. The initial hemogram revealed a marked leukocytosis and an extreme monocytosis. The patient was treated for an infection of undisclosed etiology and 5 days later it was discharged with a request that it be returned after a week for a recheck. Three blood studies on initial admission and 6 studies after return for recheck are summarized in Table 45.

A border-line anemia existed at all observations and a bone marrow aspiration on December 9 revealed 95 per cent of the nucleated cells to be similar to the abnormal cells in peripheral blood, thus demonstrat-

ing a marked displacement of bone marrow by neoplastic cells. On December 16, a flaccid paralysis of the rear limbs developed with extension of partial paralysis to the forelimbs. Death occurred on December 19. Cerebrospinal fluid drawn after death revealed 1,250 nucleated cells per cmm. and 81 per cent were large mononuclears. The necropsy report revealed accumulations of neoplastic cells in lymph nodes (architecture fairly normal) and red pulp of the spleen, extravascularly in the kidney and liver, perivascularly in the duodenum, and the femoral marrow showed a predominance of blast cells of the tumor type.

Table 45. Blood Studies in Monocytic Leukemia in the Canine

<i>Date</i>	<i>PCV</i>	<i>ESR*</i>	<i>WBC</i>	<i>Meta.</i>	<i>Band</i>	<i>Seg.</i>	<i>Lymph.</i>	<i>Pro-mono-cyte</i>	<i>Mono-cytes</i>	<i>D.C.°</i>
Nov. 17	33	54/+34	86,800	0.5	2.5	22.5	8.5	0.0	65.5	0.0
" 18	34	55/+07	52,800	5.0	7.0	39.0	6.0	0.0	36.0	7.0
" 21	33	39/+19	9,300	0.0	1.0	36.5	22.0	0.0	31.5	9.0
Nov. 30	35	38/+22	30,200	0.0	1.75	3.75	16.75	54.5	18.75	5.5
Dec. 7	33	50/+30	53,450	0.0	2.5	6.25	6.25	63.0	19.5	2.5
" 9	36	54/+40	51,400	1.75	2.0	3.75	4.25	84.25	1.75	2.0
" 12	38	30/+18	80,050	0.0	0.75	2.0	2.75	70.25	19.25	5.0
" 14	35	50/+34	103,000	0.0	0.0	0.5	4.0	72.5	21.0	2.0
" 16	34	56/+38	93,500	0.0	0.67	2.33	3.33	55.33	38.33	0.0

* Erythrocyte sedimentation rate at one hour, observed rate/corrected rate.

° Degenerated cells.

Treatment with aureomycin was associated with a fall in leukocyte count, although an absolute monocytosis persisted. After the blood study of December 7, the possibility of a monocytic leukemia became apparent. The morphology of the abnormal cells varied from one examination to another in that the nuclei were predominantly ameboid and often folded at one time (Plate X) and at another time the nuclei were essentially round so that the true nature of the cell was in doubt.

Myelomatosis. A neoplastic disease of the bone marrow involving the plasma cell. Single cases have been described by Bloom,³² Jennings,³⁷ Meier,³⁸ and Cordy.³⁴ The disease is also called multiple myeloma or plasma cell myeloma. It is not a common disease in animals or man. There is usually multiple involvement of the bones resulting in bone pain, deformity and fracture. Jennings states that in his patient the blood picture was normal with the exception of a border-line anemia.

Miscellaneous. A case of basophilic granulocytic leukemia has been reported by Romanelli⁴⁰ in Italy. A case of canine malignant lymphoma simulating Hodgkin's Disease has been described by Moulton and Bostick.³⁹ Meier³⁸ has given a brief description of 6 cases of possible Hodgkin's disease observed at Angell Memorial Hospital, Boston.

THE LEUKEMIA COMPLEX IN THE FELINE

Leukemia and related malignancies of the hematopoietic tissues of the feline have recently received considerable attention from Holzworth, Meier and co-workers at the Angell Memorial Hospital, Boston. Neoplasia involving the lymphocytic series^{41,43,44,47} predominates in the feline, as in the dog and cow, but in addition a few cases involving other cell-types have been encountered, *e.g.*, myelogenous (granulocytic) leukemia,^{41,46} reticulum cell myeloma,⁴² basophilic, eosinophilic and monocytic leukemias.⁴¹ Malignancy of one form or another involving cells of the hematopoietic tissues was found to be present in 10 per cent of 1,425 cats coming to necropsy over a 12-year period.⁴¹ From this it would appear that the leukemia complex is of considerable significance in the feline.

Differential diagnosis is complicated because an elevation of body temperature and a well advanced anemia are characteristically associated with the leukemia complex in the feline. No doubt many cases in veterinary practice have been treated as feline infectious anemia or as other forms of refractory anemia without the leukemia complex coming under consideration.

The presence of progressive anemia should lead to a thorough examination for swelling in superficial lymph nodes and the examination of both thoracic and abdominal cavities for tumor masses. Dyspnea and fluid in the chest cavity should suggest the possibility of a neoplastic mass in the thorax. Diffuse involvement of the liver leads to development of icterus. Table 46 presents the details of clinical and blood findings in 9 cases, of which 6 cases were classified as lymphosarcoma with only 2 showing a true leukemia; 2 cases were classified as granulocytic leukemic leukemias, and 1 case was thought to be erythroleukemia.¹⁴

Erythroleukemia. Case 57S380 in Table 46 designated erythroleukemia was a male cat, 5½ years old, admitted with the history of anorexia of two weeks duration. The temperature was 104° F., the mucous membranes were pale and icteric. Blood examination revealed severe anemia (PCV 10.5) and an icterus index of 40 units. The most unusual feature was a large number of nucleated erythrocytes some of which were giant forms of metarubricytes and 23 per cent of unclassified cells with a deeply basophilic cytoplasm and an eccentric nucleus (Plate X). The total leukocyte count corrected for nucleated red cells was 16,100. Despite the large number of nucleated erythrocytes, there was no evidence to substantiate normal erythropoiesis in response to the anemia. There were no reticulocytes or polychromatophilic erythrocytes in peripheral blood. A second blood examination 5 days later revealed only 5 nucleated erythrocytes per 100 leukocytes and another study 10 days later showed 65 nucleated erythrocytes per 100 leukocytes. A bone marrow aspiration

revealed 67 per cent of the nucleated cells to be similar to the unclassified cells of peripheral blood, but in addition some nucleated cells of the erythrocytic series, that could be identified by the presence of hemoglobin, were giant forms with small nuclei. The patient was put to

Table 46. Nine Cases of the Leukemia Complex in the Feline

<i>Case No.</i>	<i>Clinical Characteristics</i>	<i>Temp.</i>	<i>RBC</i>	<i>WBC</i>	<i>Comments</i>
55S2862 (6½ yr.)	Anorexia, dyspnea, enlarged nodes, icterus, cough.	—	4.42	5,150	Lymphopenia; lymphosarcoma with fluid in thoracic cavity.
55S257 (7 yr.)	Anorexia, vomiting	103	7.31	6,250	Lymphopenia; lymphosarcoma with kidney, spleen and lymph node involvement.
56S872 (9 mo.)	Anorexia, dyspnea, mass in thoracic cavity, emaciation.	101	5.42	9,450	Lymphopenia; lymphosarcoma with generalized lymphadenopathy. Thoracic mass.
56S986 (5 yr.)	Anorexia, vomiting, emaciation	104.5	2.46	12,550	14% polymorphocytes; lymphosarcoma with kidney, liver and lymph node involvement.
56S2396 (4 yr.)	Anorexia, vomiting, increased lung sounds with dyspnea.	104	4.68	118,000	99% lymphocytes; lymphosarcoma with mass in thorax and enlarged abdominal nodes.
56S1679 (9 yr.)	Anorexia, dyspnea, fluid in pleural cavity	104	2.84	65,600	99% lymphocytes most of which are polymorphocytes. Spleen enlarged.
56S1435 (5 yr.)	Enlarged lymph nodes	103.2	2.71	67,400	Many immature cells suggesting granulocytes. Possibly granulocytic leukemia.
56S1879 (2 yr.)	Slight enlargement of nodes, anorexia and depression.	104.3	1.01	78,800	33% of cells unclassified. Possibly granulocytic leukemia.
57S380 (5½ yr.)	Anorexia and icterus	104	1.19	16,100	40 to 60 nucleated RBC/100 WBC. Many unclassified cells. Possibly erythroleukemia.

death and necropsy examination revealed a liver that was paler than normal and presented many small nodules, a spleen somewhat swollen and very red, and enlargement of a mediastinal lymph node and ileocecal node. The histopathology was described as patchy infiltration of neoplastic cells mainly in the portal areas of the liver and along sinusoids and about the central vein. The neoplastic cells were noted to vary considerably in size. The spleen showed atrophy of follicles and diffuse infiltration by neoplastic cells. There was atrophy of follicles in the lymph nodes with hypertrophy of the reticulum and infiltration by neoplastic cells. The cells were described as varying in size from large irregular myeloblasts to small dark staining cells.

Basophilic Leukemia. Meier and Gourley⁴⁵ have made a detailed hematologic and pathologic report of a case of basophilic or mast cell leukemia. A picture of the neoplastic cell as it appeared in peripheral blood is identical with the cells found in the single case reported here. This rare type of leukemia was observed at termination of the disease in an 8-year-old, spayed female cat. The patient was said to have had an enlarged abdomen for about 6 months and that it had been losing weight. The cat was moribund on arrival at the clinic, the rectal tempera-



FIG. 21. View of the abdominal organs of a cat with basophilic leukemia. Lower center is occupied by the tremendously enlarged spleen and in the upper left center the liver presents multiple, small white foci.

ture was 99° F. and a markedly swollen spleen could be palpated. A blood sample was drawn by cardiac puncture shortly before death and films were prepared from the bone marrow at necropsy.

The blood findings were: RBC, 5,300,000; Hb., 7.7 grams per cent; WBC, 30,000; and the differential count revealed 35 per cent of the leukocytes with a cytoplasm densely packed with basophilic granules which suggested the mast cell more than the blood basophil (Plate X). The same type cell was present to the extent of 6.3 per cent of the nucleated cells of the bone marrow.

The essential feature of the necropsy was the tremendously enlarged spleen (Fig. 21) and the occurrence of multiple 5 mm. white foci in the liver. There was a large ulcer in the stomach mucosa. The lymph nodes were not enlarged. The histopathologic report states that in

addition to the extreme splenic involvement, the neoplastic cells were found in one small focus in the lungs and in the subcapsular sinus of a lymph node. The report did not comment on presence of the tumor cells in the liver.

THE LEUKEMIA COMPLEX IN THE EQUINE

Four cases of malignancy involving lymphocytic cells have been seen in the University of California Veterinary Clinic over a period of 10 years. Between 25 and 30 horses have been in the clinic at all times and from this it would appear that neoplasia of lymphocytic tissue is of rare occurrence in the equine. This is in agreement with Runnells and Benbrook⁵³ who observed only 6 instances of equine lymphosarcoma in a collection of more than 600 tumors of domestic animals collected over a 20-year period. A search of the foreign literature, especially the German veterinary journals covering the last 60 years, reveals several dozen references to equine leukemia, pseudoleukemia, or lymphadenosis.

The 4 cases referred to above occurred in equines 6, 10, and 13 years of age. The 6-year-old was a mare, the others were geldings. The complaints on admission were (1) skin tumors and poor condition; (2) edema of ventral abdominal wall; (3) bumps in the skin a year ago which disappeared and reappeared 2 months ago; and, (4) swollen prefemoral and cervical lymph nodes. The latter case was the only one with an above normal temperature of between 101.7 and 103.4, and this case also had a pleural effusion which at necropsy was recorded as consisting of 5-6 liters of a dark orange-brown fluid. Progressive weight loss was common to all cases.

Runnells and Benbrook⁵³ did not have blood studies with their material. Tutt,⁵² reporting on a case in a Thoroughbred stallion 9 years of age, did not observe a leukemic blood picture. Anemia was not a feature of the disease in the 4 cases described here, for hemoglobin ranged from 10.5-14 grams per cent and the PCV readings were from 29-40. Three equines had total leukocyte counts of 15,000-16,000, which is a leukocytosis, and two of these had neutrophilia of 70+ per cent and lymphocytes in the range of 14-20 per cent. The third equine of this group had 54 per cent lymphocytes, many of which were atypical in that the cells were large and the cytoplasm was deeply basophilic and often presented vacuoles. The fourth case was observed over a period of 3 months and 12 separate blood examinations were made during this period. The hemoglobin ranged from 10.5 to 13.3 grams per cent; the leukocyte counts for the first 6 weeks were between 7,700 and 8,500 after which a steady rise occurred reaching 25,000 at the time the patient was put to death. When the total count was lowest, the differential leukocyte count revealed lymphocytes to range between 18 and 47 per

cent and cells classified as monocytes ranged from 12.5 to 31.5 per cent. This latter finding was unusual and on several occasions it was noted that some of the monocytes were atypical. When the leukocyte count became elevated, this was associated with a neutrophilia reaching 70 to 73 per cent. Thus, a moderate leukocytosis with neutrophilia was ob-

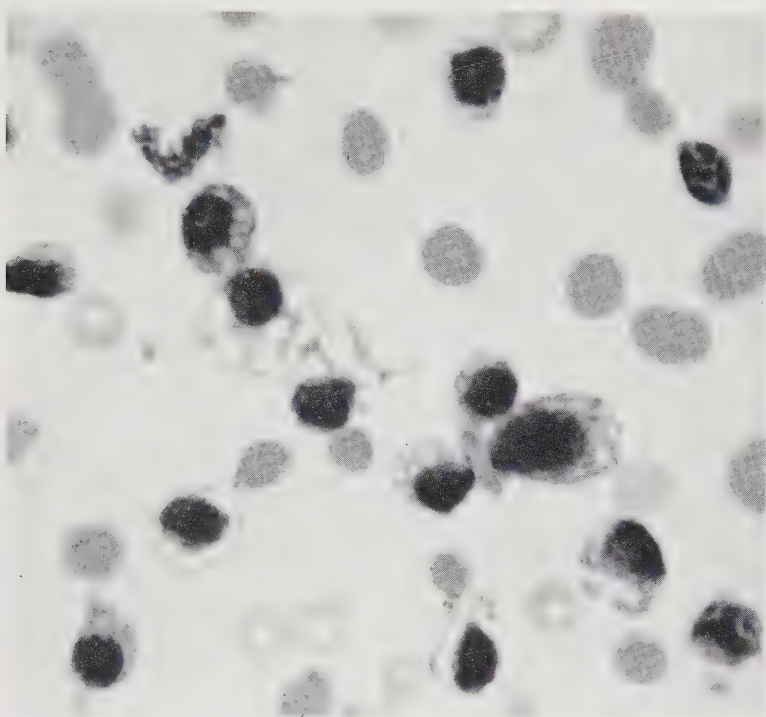


FIG. 22. Myeloma plasma cells with characteristic foamy cytoplasm in bone marrow aspirated from the tuber coxae of an equine with myelomatosis ($\times 640$). (Cornelius, Goodbary and Kennedy, courtesy of Cornell Vet.)

served in all 4 cases and in 2, the peripheral blood presented monocytoïd cells some of which had features suggesting immaturity.

Myelomatosis in the Equine. A case of plasma cell myelomatosis⁴⁹ in a 16-year-old Thoroughbred gelding was observed at the University of California Veterinary Clinic. The patient was admitted to the clinic with a history of lameness and weakness of 2 days' duration following heavy work. The right foreleg exhibited marked edema and tenderness.

Examination of the blood (Table 47) revealed a severe macrocytic anemia. The clinical condition improved after symptomatic treatment for 2 weeks although the severe anemia persisted. The patient was sent home with instructions recommending rest and improved nutrition. At

first the patient gained weight but 3 months later a partial paralysis developed and he was unable to rise. After 48 hours, the horse was able to stand again but showed irregular anorexia, loss of weight, and edema of all extremities and the intermandibular space. Other clinical signs were systolic murmur, jugular pulse, heart rate of 100 per minute, moist rales over the ventral two-thirds of the lungs, a fetid breath, and pale mucous membranes.

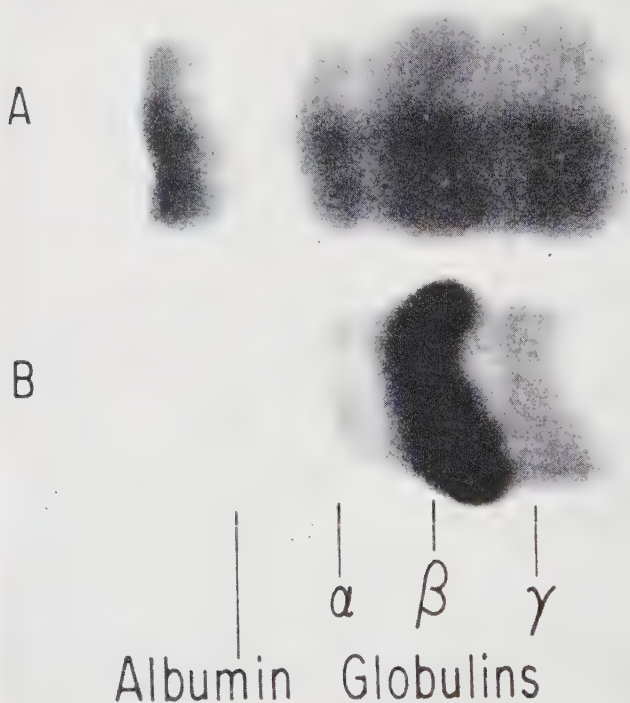


FIG. 23. Paper electrophoretic separation of equine serum proteins. A, normal control horse; B, horse with myelomatosis exhibiting an abnormal beta myeloma protein, hypoalbuminemia, and hypo-alpha-globulinemia. (Cornelius, Goodbary, and Kennedy, courtesy of Cornell Vet.)

Two examinations of peripheral blood were made prior to sacrificing the animal and bone marrow was aspirated from the tuber coxae. The anemia was more severe now and continued to be macrocytic normochromic (Table 47). The leukocyte picture was one of leukopenia and a relative neutrophilia. The bone marrow findings provided the diagnosis for myeloma plasma cells accounted for 65 per cent of the nucleated

cells of the marrow (Fig. 22). There was a marked deficiency of precursors for both the granulocytic leukocytes and the erythrocytic series.

Electrophoretic separation of the serum proteins on filter paper revealed the presence of an abnormal myeloma beta globulin, which was estimated to be 8.4 Gm. per cent. A marked deficiency of albumin and alpha-globulin was observed (Fig. 23). The Bence-Jones protein commonly found in the urine in myelomatosis was not present in urine collected at necropsy.

Table 47. Hemograms from a Case of Myelomatosis in the Equine

Date	RBC	PCV	Hb	MCV	MCHC	WBC	Meta.	Band	Neut.	Lymph.	Mono.	Eos.	Bas.	Unclass.
July 8	2.70	16.0	5.4	59	34	3,200	0.0	3.0	68.0	34.0	1.0	3.0	1.0	0.0
Oct. 13	1.13	7.5	2.5	66	33	6,000	0.0	2.0	77.0	20.0	0.5	0.0	0.0	0.5
Oct. 16	1.06	8.0	2.7	75	34	6,400	12.5	22.0	32.0	30.5	2.5	0.0	0.0	0.5

Only the femur and humerus bones clearly showed gross lesions, but by microscopic examination the ribs and vertebral column were found to be involved. All bone sections examined showed extensive replacement of normal marrow elements by myeloma cells. Although the lymph nodes were not enlarged, all sections examined microscopically showed an almost complete replacement of lymphocytes by myeloma cells of the type seen in the bone marrow.

MISCELLANEOUS CASES

Lymphosarcoma in a Goat. A 3-year-old, female Saanen-Nubian cross-bred goat was the second such case to develop among the animals of a goat dairy in Southern California. The course of the disease from first clinical signs was approximately 45 days. The patient had milked up to 10 days prior to death. One blood examination was made and a moderate anemia was demonstrated by the following; erythrocytes, 11,900,000 per cmm.; PCV, 18.5 per cent; and hemoglobin, 6.8 grams per cent. The total leukocyte count was at the high normal level of 15,100. The percentage distribution of leukocytes in the differential count was band neutrophils, 2; mature neutrophils, 31; lymphocytes, 61, of which 13 per cent consisted of large, atypical and in some instances bi-nucleate forms; and monocytes, 6.

The patient was afebrile and it maintained an appetite up to the day before death. There was open mouth breathing throughout, which became more pronounced 24 hours before death. At necropsy all lymph nodes of the body were enlarged.

Swine. Monlux, Anderson, and Davis⁵¹ in a survey of tumors occurring in cattle, sheep, and swine at Denver's Federally inspected abattoirs found 9 lymphosarcoma cases among almost one million hogs slaughtered in 1953 and 1954. Eight of the 9 hogs were estimated to be between 6 and 12 months of age and 1 was a 3-year-old. A similar incidence of approximately 1 case of leukemia per 100,000 hogs slaughtered was reported from all Federally inspected abattoirs in 1953 (Table 48). A case designated "lymphoblastoma" was reported in an aged sow weighing about 250 pounds.⁴⁸ Also, a case of lymphocytic leukemia in a hog receiving ionizing radiation has been described.¹⁵

Table 48. The Occurrence of Tumors in Three Species of Animals Slaughtered at all Federally Inspected Abattoirs in 1953 (from Monlux, Anderson and Davis⁵¹)

<i>Species</i>	<i>Number of Animals Inspected</i>	<i>Number of Carcasses Condemned at Ante or Postmortem Inspection for the Following Conditions:</i>				
		<i>Carcinoma</i>	<i>Epithelioma</i>	<i>Leukemia or Lymphoma</i>	<i>Sarcoma</i>	<i>Miscellaneous</i>
Cattle	15,208,023	1,538	4,402	1,518	540	2,247
Calves	6,027,449	14	2	100	15	89
Sheep and						
Lambs	13,623,394	51	4	34	26	131
Swine	57,395,484	241	0	529	234	624

Langham and Hallman⁵⁰ referred to a probable case of leukemia of granulocytic origin detected at slaughter of a 210-pound hog. The size of the spleen attracted the attention of the farmer and he submitted it for examination. It measured 71 cm. by 16 cm. and weighed 2 kilograms. Numerous small, yellowish-gray nodules were distributed throughout the organ. Both in the red pulp and malpighian bodies, the lymphocytic cells were largely replaced by immature cells of the granulocytic series. The most conspicuous cells were progranulocytes and myelocytes. The blood contained in vessels in the tissue sections showed characteristics of granulocytic leukemia.

Sheep. Monlux⁵¹ reports the occurrence of 19 cases of lymphosarcoma and 2 cases of myelomatosis among 1,100,000 sheep slaughtered in Denver in 1953 and 1954. A comparison of the incidence of leukemia in sheep with calves and cattle as reported from all Federally inspected abattoirs in 1953 is shown in Table 48. On the basis of these data, leukemia is of rare occurrence in sheep and lambs as compared with its occurrence in calves and cattle.

References

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Chapter 10

Blood Pictures in Some Common Diseases of Domestic Animals

It is the purpose of this chapter to outline briefly the characteristic clinical pathologic findings in certain common diseases of domestic animals. It should be recalled that laboratory data is of greatest value when combined with a complete history and a thorough physical examination. A blood study represents the situation in peripheral blood at the moment the blood is drawn and, therefore, laboratory data become increasingly more meaningful as tests are repeated to follow the progress of a disease.

CHRONIC INTERSTITIAL NEPHRITIS WITH UREMIA IN THE CANINE

This is a commonly encountered terminal disease especially in older dogs. Males were involved in 69 per cent of 49 cases and in 79 per cent of 43 cases the age was 5 or more years. Polydipsia, polyuria, vomition, oral ulceration, uremic breath, and/or pale mucous membranes are common clinical signs. A summary of 16 cases is presented in Table 49 and 2 cases (10 and 11) are detailed in the appendix. The following clinical pathological data may be anticipated: a normocytic normochromic anemia with PCV below 37 in 60 per cent of the cases; a corrected erythrocyte sedimentation rate generally falling in the +20 to +30 range; a variable total leukocyte count, rarely leukopenic, but generally in the high normal range with about 30 per cent of the cases showing total counts above 20,000; and, in older dogs, 6+ years of age, a neutrophilia with slight left shift and *absolute lymphopenia* are of common occurrence. No consistent pattern has been observed in the number of monocytes and eosinophils although a marked but temporary elevation of eosinophils has been encountered in isolated cases.

The specific gravity of the urine is low and falls below 1.016. Due to marked acidosis the CO_2 combining power falls considerably below the minimum normal of 35 volumes per cent. Non-protein nitrogen is generally significantly elevated above the high normal of 40 mg. per cent.

Holman⁴ produced renal insufficiency in the dog by injection of uranium nitrate or mercury bichloride, or by bilateral nephrectomy and

Table 49. The Hematology of Chronic Interstitial Nephritis in the Canine

Clinic No.	Age yrs.	Sex	Urine Sp. gr.	NPN mg. %	CO ₂ Comb. Vol. %	Serum Ca.	P.	Sed. Rate	PCV	Hb.	RBC	Retic. %	Differential Leukocyte Count in Per Cent				
													WBC	Band	Neut.	Lymph.	Eos.
524B	2½	M	1.012	160	—	—	—	79/+24	18	5.9	3.02	0.6	18,450	0	55.0	21.0	8.0 16.0
54S2876¹	2½	M	1.010	140	—	13.0	16.8	77/+16	16	6.8	2.71	—	18,650	7.0	66.0	16.0	11.0 0
54S2418	4	F	1.007	188	14.5	9.0	17.0	9/—2	39	14.7	6.65	—	6,350	0	70.0	17.0	11.0 2.0
55S3695²	4½	M	1.012	144	24.0	9.0	8.8	—	20	6.8	2.63	0.1	12,600	3.0	89.0	5.0	2.0 1.0
A.E.C26	5	F	—	190	—	—	—	42/+22	33	10.3	5.04	1.0	12,100	0	76.0	14.0	6.0 4.0
53S417	5	F	—	87	—	8.6	8.6	70/+32	24	7.5	3.83	—	8,900	0	69.0	13.0	13.0 5.0
54S1739	5	M	1.009	92	18.5	13.0	13.7	76/+27	20	6.2	3.04	—	12,600	0	58.0	22.0	9.0 11.0
55S3567²	5	M	1.011	269	10.0	8.0	33.4	78/+17	16	5.4	2.01	0	9,500	0	84.0	11.0	5.0 0
54S2966	5½	M	1.009	565	19.8	8.5	16.2	58/+38	33	—	—	—	17,300	1.0	86.0	4.0	8.0 1.0
54S1717	9	M	1.006	61	17.0	—	—	80/+13	14	4.0	2.07	0.4	20,200	8.0	76.0	3.0	13.0 0
55S3753³	10	M	1.018	498	—	—	—	41/+33	42	13.8	6.86	0	14,400	1.0	94.0	3.0	2.0 0
5067	11	M	1.012	192	—	—	—	—	33	11.0	5.54	—	23,700	3.0	89.0	2.0	5.0 1.0
53S872¹	12	M	1.011	135	—	8.5	12.0	79/+18	16	5.2	2.34	0.2	18,300	1.0	76.0	6.0	16.0 1.0
54S2577	12	M	1.003	126	12.2	—	—	74/+28	21	7.8	3.80	—	14,000	1.0	73.0	15.0	4.0 7.0
527B	13	M	1.010	130	—	—	—	65/+37	29	11.0	3.70	—	11,850	2.0	88.0	1.0	9.0 0
54S2963	14	M	1.006	125	11.3	10.5	14.3	66/+32	26	10.2	4.46	—	21,300	3.0	75.0	7.0	10.0 5.0

¹ Granulomatous swelling of shoulder, focal interstitial pneumonia;

² osteodystrophy;

³ urine collected at necropsy.

Table 50. *The Hemogram in Pyometra in the Canine*

Clinic No.	Age Yr.	Temp.	Vaginal Discharge	Surgical Report	NPN	PCV	Sed. Rate	Differential Leukocyte Count in Per Cent							
								WBC	Myelo.	Meta.	Band	Neut.	Lymph.	Mono.	Eos.
54S614	8	100.0	hemorrhagic	Uterus grossly enlarged	—	39	36/+25	14,350	0.0	0.0	0.0	84.5	6.0	4.5	5.0
55S1356	8	101.6	Heavy and purulent	Large 3 inch swelling right horn.	—	48	30/+28	14,350	0.0	0.0	1.0	85.0	7.0	7.0	0.0
54S2567	4	100.2	Copious and creamy	Both horns engorged, filled with pus	—	21	71/+25	16,150	0.0	0.0	9.0	43.0	19.0	28.0	1.0
56S816	5	101.6	Creamy	Uterus enlarged full of brown pus	—	37	52/+39	26,550	8.0	53.0	21.0	6.0	5.0	6.5	0.5
58S541	10	101.2	None	Spayed 8 yrs. age. 3 × ½ inch stump filled with pus	—	50	19/+19	26,800	0.0	0.0	1.0	78.5	12.5	7.0	1.0
59S19	8½	102.0	Much discharge	Uterus Enlarged. Cystic ducts.	—	48	22/+20	33,000	0.0	0.0	0.0	80.5	6.5	8.5	4.5
59S43	4	102.0	None	Both horns grossly distended with pus	—	46	29/+25	34,200	1.0	4.5	25.5	48.5	6.0	14.5	0.0

58S124	10	101.4	Thick discharge	Ulcerated endometrium with pus.	70.0	53	7/0	35,150	0.0	0.5	8.0	71.5	7.0	12.0	1.0
55S9237	1 $\frac{1}{2}$	?	Discharge 4-5 days	Pyometra, foxtail in uterus	—	39	50/+39	38,000	0.0	1.0	7.0	71.0	14.0	7.0	0.0
58S1636	7	102.2	None now	Ulcerated endometrium with pus	—	46	12/+8	38,100	0.0	0.0	0.0	83.5	8.0	5.0	3.5
54S2665	6	103.4	Copious	Both horns filled with liquid pus.	—	37	3/—10	39,150	0.0	0.0	0.0	70.0	1.0	27.0	2.0
58S923	12	102.4	Heavy purulent	Uterus full of pus	60.0	20	74/+25	45,450	4.0	14.0	24.0	55.5	1.0	0.5	1.0
57S1332	10	100.0	None	Both horns enlarged	63.0	45	27/+22	41,300	0.0	7.0	19.0	56.0	9.0	8.0	1.0
56S906	7	102.0	Discharge	Died in surgery Horns full of pus	144.0	45	1/—4	82,500	1.0	2.5	24.5	59.5	3.5	9.0	0.0
55S9527	6	103.0	None	Died. Autopsy = pyometra	97.5	34	28/+10	88,800	0.0	7.0	24.0	51.0	6.0	10.0	2.0
54255A	14	101.4	None	Both horns full of pus	—	33	—	108,000	0.0	0.0	1.0	75.0	14.0	8.0	2.0
55S9525	8	102.0	Discharge	Uterus much enlarged	70.5	39	43/+32	112,000	0.0	0.0	2.5	80.0	7.5	8.5	1.5
56S905	8	101.0	None	Horns enlarged	78.0	45	1/—4	126,000	1.0	6.0	20.0	66.5	0.5	5.5	0.5

studied the effect of uremia on the lymphocytic tissues. Gross and histologic changes interpreted as "exhaustion atrophy" were consistent findings in the lymph nodes, the spleen, thymus, Peyer's patches and solitary follicles in a group of 78 adult dogs. The lymphocyte number in peripheral blood showed a sharp reduction which averaged 32 per cent below the control level.

PYOMETRA IN THE CANINE

This is a disease of the female dog occurring most commonly after 5 years of age. A summary of 18 cases is presented in Table 50 and the details of a typical case are presented in Appendix, Case 4. Pyometra is a good example of the effect of localization of an inflammatory process on the level of circulating leukocytes. In Table 50, the cases are arranged in order of ascending leukocyte counts. Five of the 18 cases had spectacular total leukocyte counts of 82,000 to 126,000. Such high counts as well as counts associated with a moderate to marked shift to the left are to be classified as leukemoid reactions. An absolute monocytosis occurred in 77 per cent of the cases; this is to be expected in an inflammatory process such as pyometra where much tissue debris needs to be dealt with. The large accumulation of pus brings about a toxic condition and the patient exhibits some clinical signs similar to those seen in chronic interstitial nephritis, *e.g.*, vomiting, polydipsia, anorexia, depression and dehydration. The leukocyte count is often above 30,000 in pyometra, whereas counts of that magnitude are infrequent in nephritis. The erythrocyte sedimentation rate is generally elevated in pyometra as in nephritis, but a well advanced anemia is infrequent in pyometra although common to nephritis with uremia. Non-protein nitrogen may be elevated as a result of a combination of dehydration and the decomposition of the purulent exudate but the NPN rarely reaches the levels commonly seen in the uremia of nephritis.

Pyometra is considered to be a consequence of a hormone imbalance in which hyperplastic changes occur in the endometrium.^{8,9} The purulent exudate may be sterile, but it is not uncommon to isolate a mixture of bacteria such as micrococci, streptococci, *E. coli* and/or *Proteus vulgaris*. Contamination of the pus by bacteria probably takes place through the patent cervix in those cases where discharge from the vulva occurs.

HYPOTHYROIDISM IN THE CANINE

The clinical syndrome of frank hypothyroidism in the canine generally includes a gain in weight to the point of excessive obesity and this is associated with lethargy, listlessness or sleepiness. The most common

additional complaint refers to the texture of the skin and hair coat. There may be progressive alteration of the skin leading to thickening, wrinkling, scaliness in association with seborrhea which imparts a greasy feel and an undesirable odor to the skin, and sometimes there is pigmentation. These changes in texture of the skin are accompanied by bilateral thinning of the hair coat leading to alopecia. The areas most commonly involved are the abdomen and inner surfaces of the rear limbs, but the hair loss may extend to the axilla and fore-legs. In addition hair loss may occur on the back, tail, ears, eyelids, throat and face. The patient may scratch itself constantly but this is not a common complaint. In some cases, exuding lesions form which crust over and when removed the hair pulls away with the crusts.

Coffin and Munson³ have described 3 diseases involving the endocrines in which skin changes and symmetrical hair loss are seen, *i.e.*, Sertoli cell tumor of the testis, thyroid deficiency, and canine Cushing's syndrome in which there are basophil tumors of the pituitary gland and secondary cortical hyperplasia of the adrenal glands. Endocrine changes of sufficient magnitude to result in hairlessness occur in Sertoli tumor only after the tumor has reached a size where it can be palpated. Thus the sex of the patient and palpation of the testicles should aid in differential diagnosis. Canine Cushing's syndrome was associated in most cases with polydipsia and polyuria with a urine specific gravity generally well below the minimum normal of 1.016. An additional characteristic, which makes use of the hemogram, is the occurrence of lymphopenia and eosinopenia of sufficient magnitude to be diagnostic when clinical signs are suggestive. Meier and Clark⁶ have discussed the clinical features of diseases of the thyroid gland in the dog and cat.

The blood alterations in 13 proven cases of hypothyroidism are summarized in Table 51. Serum cholesterol level is elevated in hypothyroidism probably as a consequence of a decrease in rate of destruction (conversion to other substances) and elimination from the intestine.⁷ However, an elevated serum cholesterol is not specific for hypothyroidism but taken together with the clinical syndrome and the blood picture, the cholesterol level is of considerable diagnostic value. More specific diagnostic measures include (1) histologic study of thyroid gland tissue taken by biopsy; (2) measurement of the per cent uptake of I^{131} , and (3) the determination of protein bound iodine in the serum. The latter method, called PBI, is difficult to perform with consistent accuracy because such small quantities of iodine are being measured and, in addition, almost everything in the chemical laboratory is contaminated with iodine.¹ The PBI tests on the cases presented in Table 51 were conducted at a medical laboratory that specialized in the method. The results are regarded as valid and since in several instances the PBI values

Table 51. The Hemogram in Hypothyroidism in the Canine

Diagnostic Methods*										Differential Leukocyte Count in Per Cent										
Case	Sex	Age Yr.	% I ¹³¹ U ¹ plate	PBI $\mu\text{g.}/100$ ml.	Choles-terol mg. %	Thyroid Gland Atrophy	RBC	PCV	Hb.	MCV	MCHC	ESR	WBC	Band	Neut.	Lymph.	Mono.	Eos.	D.C.	U.C.
54S550	FX	5	**	**	470	+	5.7	31	10.2	54.4	32.9	+21	8,650	0	70.0	21.0	9.0	0	0	0
55S3692	MX	6	**	**	400	+	4.6	32	10.2	69.6	31.9	+26	6,900	0	73.5	23.0	2.0	1.5	0	0
56S2335	M	3.5	**	3.7	428	**	4.4	31	10.6	70.4	34.2	— 5	11,850	2.5	69.5	18.5	6.5	2.5	0.5	0
56S2475	M	8	**	1.0	420	**	3.8	29	9.5	76.3	32.7	+32	17,500	2.5	70.0	16.5	9.5	1.5	0	0
57S622	F	5	**	2.9	446	+	5.0	40	13.3	80.0	33.2	**	9,000	0.5	65.0	24.0	8.5	2.0	0	0
58S63	F	8	1.0	2.7	508	**	5.2	41	13.5	79.8	32.9	0	22,200	1.0	71.0	12.0	5.0	11.0	0	0
59S17	F	4	0	4.9	300	**	6.0	37	12.5	61.7	33.8	— 5	8,900	0	69.5	23.0	5.5	2.0	0	0
59S415	F	7	0	4.7	322	+	5.2	35	11.8	67.3	33.7	+2	21,050	0.5	69.5	20.0	7.5	2.5	0	0
59S518	F	9	1.0	5.7	260	**	6.1	41	15.0	67.2	36.6	+27	28,100	1.5	89.0	3.0	5.5	1.0	0	0
59S1420	M	5	0	3.2	1,454	+	4.6	30	9.8	65.2	32.7	+37	8,300	0	70.0	23.0	6.0	1.0	0	0
59S1248	F	5	0	5.4	740	+	4.2	30	10.0	71.4	33.3	diphasic +20	14,250	0	51.5	30.5	8.5	9.5	0	0
59S1187	MX	10	0	2.6	262	+	3.2	24	8.8	75.0	36.7	+29	36,500	4.5	81.5	6.0	7.0	1.0	0	0
59S1903	M	8	4.8	1.9	340	**	2.2	16	5.9	72.7	36.9	+16	14,000	4.0	50.0	27.0	8.0	7.0	0	4.0

* I¹³¹ uptake, normal range 10–40% in 72 hours.

PBI, protein bound iodine in serum, normal 3.0 to 7.0 micrograms per 100 ml. serum.

Cholesterol, normal 200–250 mg. per cent.

Thyroid atrophy—determined by biopsy or on tissues removed at necropsy.

** Means test not done.

disagree with other valid evidence for the existence of hypothyroidism, it is concluded that serum protein bound iodine is not as reliable in the dog as it is in man for evaluation of thyroid deficiency. In all cases reported here the diet and drug treatments were controlled to preclude false results from a system overloaded with iodine. Kaneko⁵ initiated the investigations in our clinic on the per cent uptake of I^{131} and he made the observations on the cases in Table 51. Kaneko pointed to the disparity in results between PBI and uptake of I^{131} ; he indicated that the maximum uptake in the dog is reached at 72 hours and also that the normal range for I^{131} uptake is between 10 and 40 per cent.

Obesity and progressive changes in the skin and hair coat coupled with a borderline anemia which is characteristically normocytic normochromic and associated with a variable number of leptocytes and/or erythrocytes appearing to have "punched-out" centers and, in addition, hypochromasia are strongly suggestive of hypothyroidism. The erythrocyte sedimentation rate can be expected to show a high corrected value in 50 per cent of the cases and this may be the result of pathologic alterations in the blood vessel walls.² When the corrected sedimentation rate produces a negative value, this may be the result of failure of rouleau formation because of a significant abnormality in red cell morphology (also see Appendix, Case 12 which is 56S2335 in Table 51).

The total leukocyte number is variable with some counts approaching low normal and others above 20,000. The differential leukocyte count is generally quite normal in distribution of leukocyte types. Eosinophil percentage was usually low but, in 12 of the 13 cases, the absolute lymphocyte number was always well within the normal range. This is helpful in the differentiation of hypothyroidism and canine Cushing's syndrome.³

LEPTOSPIROSIS IN THE CANINE

For the purposes of presentation of the clinical pathological data in leptospirosis, only those cases are included in Table 52 in which the diagnosis was confirmed by a positive blood serum titer of 1-1,000 or greater.

Leptospirosis is an acute septicemic disease with hemolytic tendencies. The most characteristic feature in blood morphology is a total leukocyte count of 20,000 to 50,000 with neutrophilia and slight left shift. Appendix Case 9 shows changes in the hemogram that may occur in a 24-hour period in acute leptospirosis. The data as arranged in Table 52 give the impression that later in the disease the neutrophil count drops and the monocyte count becomes elevated. The number of cases is insufficient to establish this as a rule. The corrected erythrocyte sedimentation rate is variable but generally presents a positive corrected value. Appendix Case 9 shows a change from +2 to +28 in a 24-hour period in the ESR. Leptospirosis shows a predilection for the kidney and

commonly produces an acute nephritis with uremia. A significantly elevated NPN or blood urea nitrogen is a common finding and of use in differential diagnosis.¹⁷ Due to kidney degeneration the urine is positive for albumin and the sediment contains granular casts, erythrocytes and leukocytes. Bile reaction is generally positive and indicanuria is present when tissue-wasting and debility set in.¹³ In experimental canine leptospirosis, Monlux²⁰ made the following observations: Erythrocyte count showed no significant change; leukocytes began to increase on third or fourth day reaching a peak on the sixth to eighth day and then subsided. The highest count was 38,000 and some dogs showed no

Table 52. Clinical Pathological Data in Canine Leptospirosis Confirmed by Positive Blood Serum Titer

Age	Sex	Days Sick	Temp.	NPN	PCV	I.I.	Sed. Rate	WBC $\times 10^3$	Differential Leukocyte Count in Per Cent				
									Band	Neut.	Lymph.	Mono.	Eo.
3 mo.	M	2	101.8	136	53	50	3/+3	28.2	1.0	93.0	4.0	2.0	0
5½ yr.	F	2	99.0	308	38	10	26/+14	49.5	7.0	81.0	9.0	2.0	1
4 mo.	M	3	102.6	**	43	20	18/+11	32.9	1.0	90.0	4.0	5.0	0
8 yr.	M	3	100.8	97	48	100	9/+7	24.0	5.0	81.0	7.0	6.0	1
2½ yr.	M	3	101.4	120	47	5	35/+32	25.3	4.5	87.0	4.0	4.5	0
3 yr.	M	?	101.5	**	38	5	36/+24	20.5	3.0	72.0	6.0	14.0	5
4 mo.	M	5	102.4	**	41	12	41/+32	26.1	1.0	76.0	7.0	16.0	0
9 mo.	F	8	100.6	338	42	5	14/+6	25.7	3.0	78.0	9.0	10.0	0
4 yr.	M	?	102.8	**	47	5	0/-3	22.5	0	46.5	29.0	10.0	14

leukocytosis. The erythrocyte sedimentation rate was the earliest variation, increasing as early as 12 hours after inoculation. The rate of fall was the best indicator of the progress of the disease. The data indicated the highest sedimentation rate on the fifth day with a return toward normal on the ninth day. Agglutinins for *L. icterohemorrhagiae* appeared between the fourth and seventh day and reached a peak in the third week; with *L. canicola* the agglutinins appeared between the sixth and eleventh days and peaked in the third week.

CANINE DISTEMPER

Canine distemper is an acute infectious disease of viral origin that readily becomes complicated by secondary bacterial infection. The complex is characterized by involvement of the upper and lower respiratory tracts, gastroenteritis, and in some cases nervous symptoms and skin eruptions. A specific diagnosis can be made at autopsy on demonstration of typical inclusion bodies in the cytoplasm of the transitional epithelium of the urinary tract, gallbladder and bile ducts.²⁷ A method

for making a positive diagnosis in the living animal by demonstration of inclusion bodies in the conjunctival and oral epithelium was described by Goss *et al.*¹⁵ More recently, Cello, Moulton, and McFarland¹² have described what they believe is the first demonstration of inclusion bodies of a virus disease in the polymorphonuclear leukocytes of peripheral

Table 53. Blood Morphology in Canine Distemper. Cases Selected as Positive on the Basis of the Demonstration of Specific Inclusion Bodies both in Conjunctival Scrapings and in Circulating Neutrophils

Case No.	Week of Illness	PCV	Sed. Rate	WBC $\times 10^3$	Differential Leukocyte Count in Per Cent					
					Meta.	Band	Neut.	Lymph.	Mono.	Eos. Unclass.
55S3179	1st	39	40/+29	8.0	0	0	85.5	7.5	2.0	5.0 0
59S223*	1st	56	0/0	15.3	0	2.5	84.0	3.5	10.0	0 0
59S241*	1st	41	12/+3	2.2	0	4.0	67.0	11.0	18.0	0 0
59S524	1st	47	0/-3	11.3	0	0	68.0	16.5	8.0	5.5 2.0
58S1460*	1st	56	1/+1	1.9	0	5.0	81.0	7.0	7.0	0 0
58S547	1st	30	22/-4	6.9	0	3.0	78.5	2.5	16.0	0 0
58S1353	1st	41	35/+26	51.3	1.0	2.5	69.0	9.0	17.0	1.5 0
58S966	1st	33	35/+15	10.2	0	9.0	70.0	12.5	8.5	0 0
58S960	1st	38	42/+30	17.9	0	1.0	69.0	5.0	15.5	9.5 0
57S1220	1st	45	25/+20	18.0	0	0	88.5	7.0	4.0	0.5 0
59S401	2nd	44	23/+17	8.9	0	2.5	85.5	1.0	1.0	10.0 0
59S402	2nd	51	2/+2	9.0	0	1.0	83.0	4.0	11.5	0 0.5
59S567*	2nd	27	63/+31	8.8	0	3.0	83.0	8.0	5.0	0 1.0
58S1072	2nd	42	0/-8	6.4	0	0.5	73.0	16.0	6.5	3.5 0.5
58S246	2nd	39	30/+19	7.3	0	4.0	76.0	2.0	15.0	0 3.0
58S90*	2nd	35	53/+37	12.8	1.5	26.0	50.0	5.5	17.0	0 0
59S271*	3rd	38	18/+6	11.4	0	2.0	87.5	3.5	5.5	1.5 0
58S923*	3rd	33	38/+18	11.3	0	1.5	60.0	22.0	16.0	0.5 0
58S1046	3rd	34	36/+18	13.1	0	1.5	82.5	8.5	5.5	2.0 0
58S139*	3rd	48	15/+13	9.1	0	4.5	69.5	2.5	23.5	0 0
58S751*	4th	36	60/+46	20.6	0	19.5	71.5	3.5	5.5	0 0
58S1052	4th	47	14/+11	7.0	0	1.5	79.0	6.5	12.0	1.0 0
58S1254	4th	43	13/+6	12.6	0	3.5	78.5	6.5	6.5	5.0 0
58S1389*	4th	31	2/-22	10.1	0	3.0	71.5	11.0	11.0	3.5 0
58S339	4th	34	48/+30	10.0	0	0	86.0	8.0	6.0	0 0

*Patient died and diagnosis of canine distemper was verified at necropsy.

blood in canine distemper. These inclusion bodies cannot be seen in Wright's or Giemsa-stained blood films but require a special fixing and staining procedure called Schorr's stain.

In presenting the blood morphology in canine distemper, cases were selected on the basis of having exhibited inclusion bodies both in conjunctival cell preparations and the neutrophils of peripheral blood. The data are summarized in Table 53 and the cases are listed according to the week of illness as stated by the owner. In distemper the owner often misses the first signs and, therefore, the listings represent only

approximate durations of illness. The data represent the hemograms at the time of admission to the clinic.

The blood morphology in canine distemper is non-specific for it is influenced by the stage of the disease and the presence of secondary bacterial invaders. The canine distemper virus depresses total leukocyte count whereas bacterial infections elevate it. During the viral stage and initial temperature, the leukopenia may be very severe but generally the WBC is in the range of 6–10 thousand. Total leukocyte counts in the range of high normal and up to 50,000+ have been common in distemper with complications. The differential leukocyte count is one of relative or absolute neutrophilia with slight left shift. In rare instances the left shift may consist of a relatively high incidence of band neutrophils and this is probably an unfavorable sign. An *absolute lymphopenia* is characteristic of canine distemper. The differential expressed in percentage gives the impression that monocytosis is a common finding but this is more relative than absolute. Only 20 per cent of the cases in Table 53 represent instances of absolute monocytosis. An anemia may develop as the disease progresses but evaluation of the red cell mass is somewhat masked by dehydration. Two of the 25 cases showed marked dehydration in the first week of illness (PCV 56) and both cases died. One-third of the cases had PCV readings below the minimum normal of 37 per cent. Sedimentation of erythrocytes usually revealed a significant corrected positive value, but a few cases had corrected negative values or low corrected positive values.

Death occurred in 10 of the 25 cases listed in Table 53. No consistent blood picture was apparent among the cases that succumbed but the following were observed: eosinopenia, all cases; marked dehydration, 2; marked leukopenia, 2; absolute monocytosis, 2; a high level of band neutrophils, 2; and well advanced anemia, 1 case. All cases that died came to autopsy and the existence of canine distemper was verified by the pathologist. Survival was observed in cases with 5 per cent or more eosinophils.

INFECTIOUS CANINE HEPATITIS

An acute infectious disease that may affect dogs of all ages but due to the widespread nature of the virus this disease, like distemper, is generally seen in young dogs. In the early stages the disease may be confused with distemper and leptospirosis. Some very suggestive signs are acute onset, swollen red tonsils, pain on pressure over the liver, and high temperature in the early stages reaching 105°–106° F. Other signs are pronounced apathy or lethargy, anorexia, abnormal thirst, vomiting which may become blood stained terminally, icterus may develop but it is of rare occurrence, and during the recovery period a

kerato-conjunctivitis in one or both eyes develops in about 30 per cent of the patients. Death may occur suddenly in 3 or 4 days or recovery may be rapid. Specific diagnosis can be made at necropsy on demonstration of intranuclear inclusions in liver cells.^{11,14,24,26}

When liver damage is extensive there is a marked prolongation of bleeding time. This begins usually on the third day of fever and persists throughout the febrile period. Liver damage interferes with prothrombin and fibrinogen production, while at the same time heparin is liberated by damaged liver cells. Bleeding time is determined by shaving a

Table 54. Blood Morphology in Suspected Canine Infectious Hepatitis. Cases Selected on the Basis of Acute Illness with Tonsillitis and/or Abdominal Pain

Case No.	Age in Months	Sex	Temp.	PCV	I.I.	Sed. Rate	WBC $\times 10^3$	Differential Leukocyte Count in Per Cent					
								Band	Neut.	Lymph.	Mono.	Eos.	U.C.
56S1864	2	M	103.0	40	-5	3/-7	7.7	0	28.5	44.5	7.0	8.0	12.0
57S1735	21	F	105.4	60	-5	0/0	7.2	7.5	65.5	16.5	9.5	1.0	0
57S1800	8	F	103.4	43	5	2/-5	1.8	0	54.0	39.0	6.0	1.0	0
58S343	22	M	104.0	46	-5	6/+2	7.0	3.0	58.5	18.0	12.5	2.0	6.0
58S909	8	F	106.0	43	-5	13/+12	4.9	3.0	0	9.0	77.0	0	11.0
58S760	1½	M	104.0	37	-5	7/-6	4.9	0	59.0	19.5	17.5	2.5	1.5
56S2069	30½	F	104.5	48	-5	20/+18	3.8	1.0	82.0	13.0	3.0	1.0	0
58S343	22	M	104.0	46	-5	6/+2	7.0	3.0	58.5	18.0	12.5	2.0	6.0
58S760	1½	M	104.0	37	-5	7/-6	4.9	0	60.5	19.5	17.5	2.5	0
58S1896	2	M	102.5	37	-5	0/-13	6.8	0.5	81.0	10.5	8.0	0	0
59S1953*	2½	F	103.6	36	-5	14/0	6.0	0	68.0	14.0	17.0	1.0	0
59S1954*	2½	F	103.4	35	-5	3/-13	9.5	0	70.5	16.5	12.5	0.5	0

*Litter mates

small area over the marginal ear vein and puncturing the vessel. The first drop is blotted immediately and thereafter every ½ minute. Average bleeding time for normal dogs is 1-2 minutes for hepatitis dogs it may be 3-45 minutes.²³

Twelve cases of suspected infectious canine hepatitis have been assembled in Table 54. The blood findings represent the picture as found in each case upon presentation of the patient to the clinic. Acute illness, high temperature, tonsillitis and/or liver pain were used as a basis for selection of the cases. None of these cases died, therefore, the data are lacking in respect to verification of the diagnosis by liver inclusion bodies. Points of interest are (a) icterus index, normal; (b) the erythrocyte sedimentation rate shows a high level of negative corrected values; (c) a severe to moderate leukopenia exists with a mean total leukocyte count of about 6,000; (d) the leukopenia is due to neutropenia and lymphopenia whereas monocytosis is for the most

part relative; and, (c) one-third of the cases showed a significant level of unclassified cells.

Liver damage interferes with production of plasma proteins (fibrinogen) and, therefore, erythrocyte sedimentation is generally less than the anticipated normal for the PCV. ESR could well be an important laboratory tool for presumptive differentiation between I.C.H. and other diseases in the canine. The leukopenia is to be anticipated and secondary bacterial infection is not the confusing problem in this disease as it is in canine distemper. The high level of unclassified cells may be related to the release of young lymphocytes during the recovery phase. Hodgman¹⁶ made the statement that leukocytosis with lymphocytosis associated with a marked increase in young lymphocytes develops after the sixth day. In experimental infectious canine hepatitis in 17 dogs, Smith²⁶ observed a decrease in number of leukocytes to less than one-half of the normal number and found lymphopenia to be an outstanding feature. These blood changes commenced from 1 to 5 days after exposure, persisted for 24 to 48 hours, and this was followed in most cases by a rapid return to normal leukocyte values. McSherry and Smith¹⁹ observed in 2 dogs infected experimentally that during recovery a marked leukocytosis occurred involving both the neutrophils and the lymphocytes and total counts were between 20 and 40 thousand before a return to normal took place.

Other clinical pathologic features in infectious canine hepatitis are albuminuria and sometimes bilirubinuria. The disease is spread primarily through the urine, and the virus has been isolated from urine for as long as 161 days after inoculation of susceptible animals.²²

INFECTIOUS FELINE PANLEUKOPENIA

A highly contagious virus disease of young cats characterized by extreme leukopenia and a high mortality rate. In the experimental disease the incubation period was found to be 4 to 8 days.²¹ There is a progressive fall in circulating leukocytes with a precipitous drop on the day of crisis. The average daily leukocyte count of 669 susceptible cats experimentally infected was as follows:²¹

	<i>Number of Cats</i>	<i>Average WBC</i>
Day of sacrifice	669	2,897
One day before sacrifice	591	9,187
Two days before sacrifice	473	11,119
Three days before sacrifice	311	11,945
Four days before sacrifice	93	12,512

In this study it was observed that the virus produced a significant swelling of both the nuclei and nucleoli of epithelial cells of the intestinal mucosa and lymphoid reticulum cells. However, the first tissue changes were

observed in the bone marrow and were characterized by diminution of cellular elements, vacuolation and apparent replacement with fat. The degree of aplasia varied with the individual and in some instances only a small cellular loss was seen, whereas in others there was almost complete obliteration of the cellular elements. Lawrence *et al.*¹⁸ describing the disease under the name of infectious feline agranulocytosis stated that the average total leukocyte count for 113 cats at the height of the

Table 55. *Blood Morphology in Feline Panleukopenia*

Case No.	Age (mo.)	Temp.	Major Clinical Signs	PCV	WBC	Differential Leukocyte Count in Per Cent*							
						Meta.	Band	Neut.	Lymph.	Mono.	Eos.	U.C.	D.C.
57S2033	3	—	Vomiting, depressed, died	—	150	0	0	2.0	18.0	78.0	2.0	0	0
57S1883	6	—	—	27	900	0	0	2.0	54.0	14.0	30.0	0	0
57S2091	36	106	Vomiting, sick 4 days	39	2,200	0	0	53.0	17.0	9.0	6.0	7.0	8.0
57S2082	17	105	Very depressed	41	1,500	0	0	10.0	80.0	6.0	4.0	0	0
57S2200	6	104	Anorexia, vomiting	36	600	0	0	0	54.0	4.0	42.0	0	0
57S1416	3	—	Anorexia	37	1,500	0	0	16.0	80.0	4.0	0	0	0
59S1948	8	105.8	Depressed, vomiting	30	700	0	2	28.0	45.0	10.0	0	4.0	11.0
59S1305	5	103.8	Vomiting, anorexia	—	1,800	14	18	38.0	28.0	2.0		0	0
60S1790	5	106.5	Vomiting, depressed	30	1,500	0	1	0	74.0	8.0	17.0	0	0
60S1730	6	105	Vomiting depressed	35	1,000	0	1	5.0	86.0	7.0	1.0	0	0
60S2153	6	—	Depressed	39	600	0	0	2.0	72.0	18.0	8.0	0	0

*U.C. unclassified cells; D.C. degenerated cells.

disease was 1,350 and that 22 cats had counts of from 0 to 200 per cmm. of blood. The findings have been much the same in our clinic as shown in Table 55. Due to the very marked reduction in neutrophils, the other leukocytes appear completely out of proportion to their true significance in the disease. In cats that survive, the leukocyte count rapidly increases after crisis and may exceed the maximum normal level for total leukocyte count in 3 or 4 days.²⁵ The leukocytosis is essentially due to neutrophilia accompanied by a shift to the left.

The major clinical signs based on 574 cases diagnosed as feline panleukopenia at the Angell Memorial Hospital¹⁰ were anorexia, 80.1 per cent; emesis, 67.9 per cent; prostration, 50.7 per cent; and, diarrhea, 24.0 per cent. Feline infectious anemia and neoplasia involving the hemato-poietic organs (leukemia) are two febrile conditions in the feline that need to be considered in differential diagnosis. The marked leukopenia

without frank anemia should distinguish panleukopenia from the two other named diseases.

TRAUMATIC GASTRITIS OR RETICULITIS IN THE BOVINE

An early diagnosis in traumatic lesions of the bovine stomach is important so that the foreign body may be removed before extensive peritonitis with abscess formation or pericarditis complicate the condition. Symptoms common to traumatic gastritis are anorexia, marked reduction in milk secretion, sluggish rumen activity, normal to slightly elevated body temperature, and evidence of pain (grunt) upon applying pressure over the xiphoid region. Many of these same signs are common to simple indigestion and, therefore, any aid that the laboratory may give should be helpful. Sixteen cases of traumatic reticulitis proven by surgical procedure are summarized in Table 56. With 5 of the 16 cows, the total leukocyte count was within the normal range and with 2 of these 5, the differential neutrophil count was not over 50 per cent. Thus, in 2 cases the leukocyte picture was not sufficiently altered by the lesion to be helpful in making a diagnosis. A neutrophilia above 50 per cent or band neutrophils in excess of 2 per cent, irrespective of total count, should be regarded as evidence of a stress reaction in the bovine. Generally, as pus formation occurs, with adhesions or abscess formation, the total leukocyte count exceeds 12,000 and may reach the 20,000 to 25,000. The single case of pericarditis in this group had a low total count of 6,100 but a marked shift to the left. It is more common with the complication of pericarditis to have a significantly elevated total count as well as a left shift. A word of caution should be introduced to the effect that a marked shift to the left does not necessarily mean traumatic reticulitis in a digestive system problem in the bovine. The following is a case wherein the hemogram could have been misleading. A mature Guernsey had gotten into the barley bin and the owner estimated she had eaten about 80 pounds of barley. Examination of the animal showed elevated heart and respiratory rates, a temperature of 100.4° F., and no rumen motility. There were no clinical signs of a foreign body so the cow was treated for acute gastric indigestion. A routine blood sample revealed the following rather surprising differential leukocyte count:

	<i>3rd day of illness</i>	<i>15 days later (recovered)</i>
PCV	49*	32
WBC	7,750	9,300
Metamyelocytes	4.5	0
Bands	22.5	0
Neutrophils	33.5	31.0
Lymphocytes	28.5	51.0
Monocytes	10.5	12.0
Eosinophils	0.5	6.0
Erythrocytes show moderate rouleau formation		

*Indicates hemoconcentration

Table 56. Blood Morphology in Traumatic Reticulitis in the Bovine

Case No.	Age	Temp.	Nature of the Lesion	P(V	I.I.**	WBC	Differential Leukocytes Count in Per Cent						
							Mye.	Meta.	Band	Neut.	Lymph.	Mono.	Eos. Bas. D.C.
FG-941	Mature	99.6	2 penetrating wires.	34	5	9,950	0	0	0	59.5	34.5	2.0	4.0 0 0
SGC809	2½ yr.	101.4	Penetrating wire, 6 days before pus in abdominal (surgery) cavity.	36	10	9,600	0	0	6.5	37.0	53.0	3.5	0 0 0
			3 days before	36.5	7.5	12,650	0	2.0	26.0	44.0	25.5	2.5	0 0 0
FG-064*	1½ yr.	101.0	One penetrating wire.	36	5	10,300	0	0	1.5	27.5	54.0	7.0	9.5 0.5 0
57L1157*	7 yr.	102.0	Several penetrating foreign bodies.	—	—	9,800	0	0	0	46.5	48.5	4.0	1.0 0 0
56L69	7 yr.	102.0	1 penetrating wire, pericarditis.	37	12	6,100	1.0	6.5	38.5	15.0	27.5	4.5	1.0 0 6.0
UCD141	Mature	102.3	3 penetrating wires, adhesions.	31	10	15,450	0	0	0	59.5	33.0	2.5	5.0 0 0
FG-094	3 yr.	103.0	Embedded wire, adhesions.	36.5	25	16,500	0	0	0	60.5	34.0	3.0	2.5 0 0
56L1481	4 yr.	101.4	2 penetrating wires.	36	25	17,050	0	0	1.5	56.5	28.0	7.0	7.0 0 0
56L718	5 yr.	—	Penetrating wire removed.	32	10	13,900	0	0	1.0	77.0	15.0	6.0	1.0 0 0
56L45	2½ yr.	102.4	Wire penetrating.	29	15	15,900	0	0	1.5	60.5	30.5	5.0	1.0 1.0 0.5
57L495	2 yr.	103.2	Penetrating nail.	25	5	14,650	0	0	1.5	40.0	33.5	19.0	0.0 0.5 5.5
FJN239	Mature	104.0	Penetrating wire, adhesions.	30.5	10	19,150	0	0	4.5	69.5	15.5	7.5	2.0 0 1.0
FJN226	Mature	103.4	Penetrating wire, adhesions.	31	5	23,650	0	0	2.0	86.5	8.5	3.0	0 0 0
56A637	6 yr.	101.0	Penetrating wire.	26	20	21,000	0	0	0	72.5	24.5	1.0	2.0 0 0
UCV962	Mature	102.3	Wire, abscesses, adhesions.	25	10	25,500	0	0	0	75.5	19.0	5.5	0 0 0
UCD149H	4 yr.	102.1	2 penetrating wires, adhesions.	40	—	21,600	0	0	0	71.0	24.0	4.5	0.5 0 0

*Leukocyte picture within normal range. **Icterus index.

Kingrey³¹ has reported on experimental bovine traumatic gastritis and gives a number of references. Of 30 foreign bodies administered to 10 cows, 25 of the objects were found in the reticulum and 19 were puncturing the wall of the organ. The most reliable of the many possible early clinical manifestations were listed as elevated temperature, *neutrophilia*, disturbed appetite for grain, pain in the area of the xyphoid cartilage, suppression of milk flow, atony of the rumen, and constipation. Arthur²⁸ summarized the leukocyte picture as follows: in traumatic reticulitis, the WBC varied between 7,000 and 12,000 with young neutrophils 8–38 per cent; in traumatic pericarditis, the total counts were 17,850 to 31,000 with immature neutrophils 22 to 66 per cent.

ACUTE BOVINE MASTITIS

Acute mastitis in the bovine may be caused by a variety of bacterial organisms but the most common are *Micrococcus pyogenes*, *Escherichia coli*, *Aerobacter aerogenes*, and *Pseudomonas aeruginosa*. *M. pyogenes* is generally responsible for the gangrenous types of mastitis although it is commonly associated with a mild chronic mastitis. Cows with acute mastitis are generally toxic and depressed and the blood picture taken early in the disease, that is during the first 24–48 hours, often is one of marked leukopenia (Table 57). The differential leukocyte count reveals a pronounced neutropenia and the lymphocyte percentage appears essentially normal, but this is relative since there is also a moderate lymphopenia. By the third day the neutrophils begin to return and this is generally accompanied by a well defined left shift. The left shift is especially quite prominent in gangrenous mastitis. The literature on the hematology of mastitis is very limited. Pattison *et al.*³⁶ in experimental mastitis due to *Streptococcus agalactiae* in goats remarked that following inoculation there was a severe leukopenia and concomitant shift to the left. Holman³⁰ in discussing the blood picture of the cow made the statement that a marked leukopenia is seen over a short period in experimental mastitis, the leukocyte count falling to about 2,000 with a return to normal in a few hours. More recently Cole and Easterbrooks²⁹ have reported on the leukopenia of acute mastitis. Theilen, Schalm, Straub and Hughes³⁸ made observations on the hematology of acute mastitis in 62 cows of which 8 cases were gangrenous. Data on representative cases of acute bovine mastitis are summarized in Table 57.

The general pattern of leukocyte response in acute mastitis appears to be as follows: within hours of the onset, a depression of both neutrophils and lymphocytes takes place. If this depression is great enough the total leukocyte count may fall below the minimum normal of 4,000 per cmm. of blood. One count as low as 1,300 (Table 57) was recorded. In other cases, the total leukocyte count remains within the normal range,

Table 57. Hemograms in Representative Cases of Acute Bovine Mastitis

				Percentage Distribution of Leukocyte Types								Comments on Clinical Examination					
Case No. and Breed	Age (yr.)	Type of Infection	Date	WBC	Myc.	Mdta.	Band	Neut.	Lymph.	Mono.	Eos.	Bas.	Temp. °F.	Pulse	Respira- tion	Other	
GTS-6 Jersey	8	<i>A. aerogenes</i>	3/ 8/57	1,300	0	0	1.0	19.0	77.0	3.0	0	0	108.8	108	58	Severe, responded to 2 treatments.	
		<i>M. pyogenes</i>	3/ 9/57	2,850	0	0	0	6.0	63.5	24.5	6.0	0	102.2	80	28		
SD-520 Guernsey	5	Sterile Sample	10/22/56	2,800	0	0.5	10.0	5.5	62.0	9.0	13.0	0	101.5	86	25	Moderately severe; re- sponded to two treat- ments.	
			10/24/56	8,000	0	2.0	14.0	44.0	27.0	9.0	3.0	1.0	101.0	75	25		
UCD-127 Holstein- Friesian	14	<i>E. coli</i>	10/ 2/56	2,950	0	2.0	5.0	2.0	66.0	17.0	8.0	0	104.8	100	100	Severe, responded slow- ly, treated for 2 weeks continuously. Liver biopsy showed micro- abscesses.	
			10/ 3/56	3,950	0	3.0	9.5	4.0	63.5	18.0	2.0	0	104.4	100	80		
			10/ 8/56	4,450	0	0	12.0	39.0	36.0	11.0	2.0	0	104.1	90	60		
			10/15/56	9,500	0	0	0.5	51.5	24.5	23.5	0	0	105.5	96	80		
			10/23/56	8,350	0	0	1.5	50.5	23.0	13.5	11.0	0	103.0	86	24		
			11/23/56	7,050	0	0	0	35.0	50.0	5.0	10.0	0	101.4	80	24		
UCD-65 Jersey	10	<i>E. coli</i>	3/12/57	1,800	0	0	1.0	2.0	83.0	12.0	2.0	0	105.0	100	*	Severe; did not respond to treatment. Liver biopsy showed severe lipidosis. Animal died.	
			3/13/57	1,350	0	0	0	1.0	85.0	6.0	8.0	0	104.2	*	*		
			3/14/57	2,850	0	0	0	1.0	82.0	8.0	9.0	0	104.8	*	*		
			3/15/57	3,250	14.0	20.0	5.0	0	51.0	7.0	3.0	0	100.0	*	*		
			3/16/57	6,300	0	7.0	0	0	84.0	9.0	0	0	100.0	*	*		
			3/18/57	7,800	4.0	23.0	8.0	7.0	51.0	7.0	0	0	101.4	*	*		
FJH-451 Holstein- Friesian	3	<i>M. pyogenes</i> (gangrene)	5/20/58	5,600	0	1.0	7.0	33.0	50.0	0	9.0	0	103.0	80	32	Two quarters gangren- ous; responded to anti- biotics and mamme- ctomy.	
			5/21/58 ¹	8,250	10.0	17.5	4.5	13.0	40.0	7.0	8.0	0	101.5	100	28		
			5/21/58 ²	10,700	5.5	31.5	8.5	14.0	34.0	6.0	0.5	0	*	*	*		
			5/22/58	9,000	3.5	5.5	23.0	24.5	36.0	3.5	4.0	0	100.2	70	28		
			6/ 6/58	5,100	0	0	0	20.0	67.0	10.0	3.0	0	101.3	70	28		
UCVA	6	<i>M. pyogenes</i> (gangrene)	10/ 2/57	4,000	22.5	22.0	15.0	2.0	28.5	10.0	0	0	100.0	180	80	Severe gangrenous mas- titis. Cow died in 24 hours.	
																¹ A.M.	² P.M.

¹ A.M. ² P.M.

but in all probability, it is considerably lower than the normal for the particular animal in question, and thus a relative leukopenia exists.

The leukopenic state persists from a few hours to a few days, depending on the severity of the mastitis and the success of the treatment. The neutrophils reappear, with a variable number of immature forms being released to peripheral blood. The extent of this shift to the left is related to the severity of the mastitis. Thus, the shift is generally more extreme in gangrenous mastitis than in non-gangrenous acute mastitis. With the appearance of the shift to the left, the total leukocyte count increases although it seldom exceeds the maximum normal total leukocyte count. This blood picture gives the impression of a degenerative left shift, but such a leukocytic pattern in acute bovine mastitis does not necessarily indicate a grave prognosis. The differential leukocyte count returns to normal in a few days or a week as the generalized clinical signs subside. In animals that recover more slowly, the shift to the left persists for a longer period.

EFFECT OF PARTURITION AND RETENTION OF FETAL MEMBRANES ON THE HEMOGRAM OF THE BOVINE

Straub, Schalm, Hughes and Theilen³⁷ have investigated the blood picture at parturition and the changes occurring with retention of the fetal membranes (Table 58 and Fig. 24).

The normal mean total leukocyte count for cattle not under stress is about 8,000 per cmm. of blood. Reports in the literature^{32,33,35} reveal a gradual increase in leukocyte number in the last weeks of gestation, with a marked rise on the day preceding parturition and a second significant increase on the day of calving. Leukocytic numbers fall sharply in the first 24 hours post-partum, with a more gradual decline thereafter, reaching normal levels on days 4 to 6 post-partum.³³

The leukocyte count in 11 cows at the moment of delivery of the calf (Table 58, Group B) was 11,800 to 24,700 with a mean of 17,700. In 24 hours the range had fallen to 5,700 to 18,000 with a mean of 10,000 per cmm. of blood (Table 58, Group C). The stress pattern was evident in the differential count for there was a marked increase in neutrophils and a fall in eosinophils (Fig. 24). The mean lymphocyte value was above the normal for cows not under stress, but since observations were not made on the differential leukocyte count on the day prior to parturition, it could not be determined from the data whether the lymphocyte count was dropping or increasing. A fall in lymphocytes is to be anticipated in the response to stress. In 24 hours, a spectacular fall in neutrophils and lymphocytes occurred associated with a slight rise in eosinophils.

Blood studies on the second day post-partum among cows with fetal

membranes dropped and fetal membranes retained (Table 58 and Fig. 24, Groups D and E) show in the latter condition a depression of total leukocyte count as well as depression of all leukocyte types, with greatest effect being on the neutrophils. In addition, a shift to the left appeared

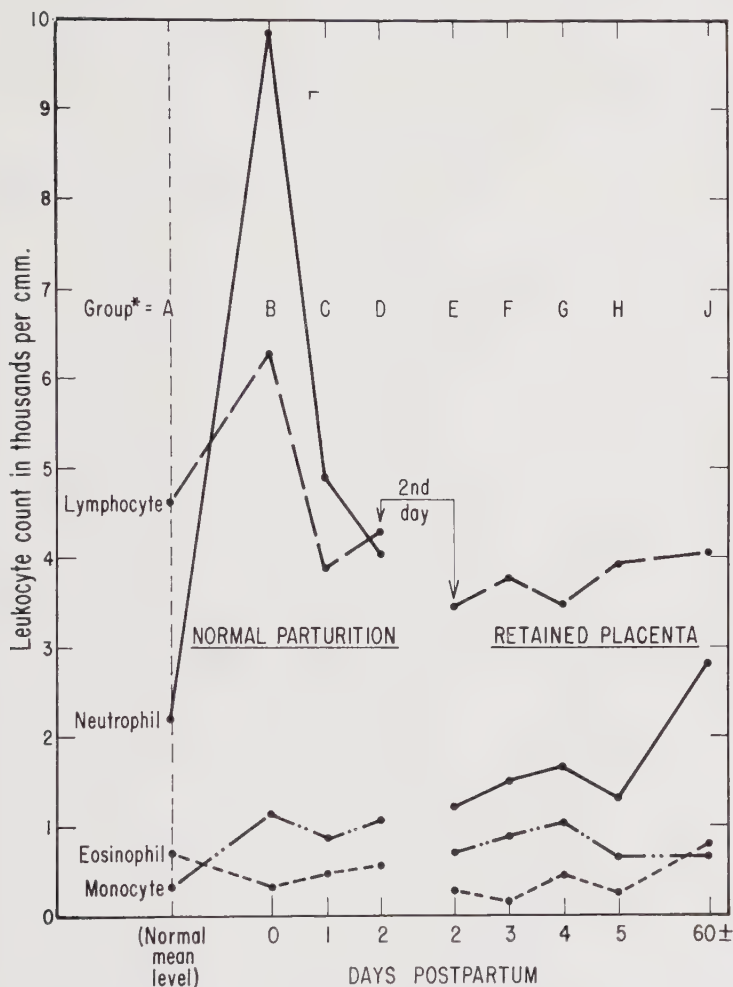


FIG. 24. Absolute leukocyte numbers as influenced by normal parturition or parturition with retained placenta. *See Table 58 for the description of Groups A through J.

with band and metamyelocyte neutrophils entering peripheral blood from the second to the fifth days. The absolute monocyte count was elevated at all times and this may be the result of the need for special enzyme systems of monocytes in the removal of tissue debris associated

Table 58. Effect of Parturition and Retention of the Fetal Membranes on the Hemogram in Cattle

Group Identification	History of the Blood Samples	RBC $\times 10^6/\text{cmm.}$	Hb. Gm. /100 ml.	PCV $\times 10^3/\text{cmm.}$	WBC $\times 10^3/\text{cmm.}$	Differential Leukocyte Count in Per Cent						
						Meta.	Band	Neut.	Lymph.	Mono.	Eos.	Bas.
A	Normal range and (mean).	5.0-10.0 (7.0)	8.0-14.0 (11.0)	24-48 (35)	4.0-12.0 (8.0)	0	0.0-2.0 (0.5)	15-45 (28.0)	45-75 (58.0)	2.0-7.0 (4.0)	2.0-20.0 (9.0)	0-2 (0.5)
B	Blood drawn at parturition (11 cows)	5.5-9.3 (7.4)	8.7-15.3 (13.2)	24-44 (40)	11.8-24.7 (17.7)	0	0.0-2.0 (0.8)	13-68 (55.4)	25-67 (34.6)	2.0-12.0 (7.4)	0.0-9.0 (1.8)	0
C	One day postpartum, mem- branes dropped. (12 cows)	6.0-8.9 (7.1)	11.8-15.4 (13.4)	35-45 (40)	5.7-18.0 (10.0)	0	0.0-3.5 (0.9)	29-61 (47.3)	28-59 (39.8)	2.5-11.5 (7.7)	1.0-12.0 (3.9)	0-1
D	2 days postpartum, membranes dropped. (8 cows)	5.6-6.8 (6.2)	10.5-14.3 (12.6)	32-43 (37)	4.9-14.7 (10.0)	0	0.0-3.0 (0.9)	15-55 (38.6)	26-61 (41.8)	6.0-16.0 (10.9)	0.5-17.0 (7.2)	0-2 (0.5)
E	2 days postpartum, membranes retained (21 cows)	4.9-12.0 (6.9)	9.5-21.0 (12.1)	30-68 (38)	3.3-9.2 (5.7)	0.5 (0.6)	0.0-24.0 (6.0)	3-35 (14.5)	30-87 (61.3)	1.0-32.0 (11.7)	0.0-21.0 (5.3)	0-2 (0.3)
F	3 days postpartum membranes retained (32 cows)	4.9-7.9 (6.4)	9.6-14.9 (11.7)	28-46 (34)	3.4-14.6 (6.6)	0.4* (1.0)	0.0-22.0 (7.5)	2-46 (17.5)	24-90 (55.9)	2.0-29.0 (13.9)	0.0-10.0 (3.7)	0-2 (0.3)
G	4 days postpartum, membranes retained (7 cows)	4.9-7.1 (6.1)	8.8-11.9 (10.6)	28-36 (33)	3.4-9.4 (6.7)	0.3 (0.7)	1.0-10.0 (4.3)	5-47 (17.6)	28-60 (55.7)	2.0-38.0 (15.0)	0.0-32.0 (6.7)	0
H	5 days postpartum, membranes retained (7 cows)	5.8-10.0 (7.1)	10.6-11.9 (11.3)	32-37 (35)	3.6-8.0 (6.1)	0	0.0-13.0 (2.7)	4-25 (20.3)	41-89 (62.2)	2.0-26.0 (10.0)	1.0-8.0 (4.8)	0
J	About 2 months postpartum following retained membranes (10 cows)	5.8-8.2 (6.5)	10.9-13.3 (11.6)	31-42 (36)	6.0-11.1 (8.5)	0	0.0-4.0 (0.5)	17-46 (34.7)	30-66 (47.7)	2.0-10.0 (7.2)	0.0-26.0 (9.9)	0-1 (0.2)

* myelocytes were also present in 4 cows in 0.5 to 2.0 per cent of the differential count.

with retained placenta. Monocytosis in retained fetal membranes also has been reported by Moore.³⁴ Winqvist³⁹ studied the bone marrow in retained fetal membranes and found a highly significant maturation inhibition within the neutrophil series with a very low percentage of mature neutrophils.

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Appendix

Case 1. Peritonitis from Gunshot Wound in the Canine

A 2-year-old, crossbred, male terrier had been noticed to be sick the night before presentation and the owner suspected poisoning since the dog was known to dig for gophers.

Physical examination showed the patient to be depressed and obviously uncomfortable as evidenced by restlessness and frequent shifting of position. Abdominal palpation elicited no evidence of pain. The temperature at the time of admission was 101.6° F. On the second day of hospitalization, the dog was vomiting, very depressed and becoming increasingly more dehydrated. The tentative diagnosis was leptospirosis. The following clinical pathological data were determined.

		Urine	
Erythrocytes	8,980,000	Transparency	cloudy
Hemoglobin	21.2	Color	dark amber
PCV	60.0	Specific gravity	1.037
MCV	66.8	pH	5.5
MCHC	35.3	Protein	two plus
Sedimentation rate	No fall in 60 minutes	Indican	four plus
Icterus index	(hemolyzed)	Bile	four plus
Nucleated erythrocytes	0.5/100 WBC	Urobilinogen	four plus
Marked rouleau formation, occasional polychromatophilic RBC. Bands and neutrophils show toxic vacuolation.		Occult blood	negative
Leukocytes	9,500	Chlorides	< 1.0 gram per liter.
Metamyelocytes	1.5	Sediment:* 3-4 coarsely granular casts/HDF, occasional cellular and fatty casts, 1-2 WBC/HDF, rare RBC.	
Bands	26.5	*(HDF = high dry field)	
Neutrophils	56.0	Leptospira not observed in dark field.	
Lymphocytes	13.0		
Monocytes	3.0		
Non-protein nitrogen	69.0 mg. per cent.		

On the third day vomiting was controlled. An abdominal radiograph was ordered. This revealed an air-gun pellet lodged in or near the colon. Physical examination revealed a small circular wound in the right flank. On the following day an intestinal anastomosis was performed. The dog did not respond and died shortly after completion of surgery.

The necropsy diagnosis was trauma due to gunshot. There were, in addition, kidney lesions consistent with a concurrent leptospiral infection.

Interpretation:

1. PCV of 60 in the canine indicates hemoconcentration.
2. Sedimentation of erythrocytes precluded by the high PCV value.
3. Marked increase in immature neutrophils in the absence of leukocytosis indicates a grave prognosis and is referred to as a *Degenerative Left Shift*. This finding is consistent with septicemia originating from the torn intestine.

4. Total WBC is bordering on leukopenia when the existence of hemoconcentration is taken into consideration. Bringing the patient into water balance would reduce the WBC by 1/4 or to about 7,000 per cmm.
5. There is an absolute eosinopenia.
6. Proteinuria and cast formation from kidney stress and bilirubinuria and increased urobilinogen levels in the urine from leptospiral hepatitis were of special interest.
7. The low chloride level was due to protracted vomiting producing primary chloride deficit and renal conservation of chloride.
8. High indican level in the urine suggests a possible intestinal involvement. (putrefaction).

Case 2. Kidney Abscess in the Canine

A 9-year-old, male Cocker Spaniel was admitted to the Clinic August 30 with a history of poor appetite for one week. No vomiting had been noted; no history was obtained of water intake or of the frequency of urination.

Clinical examination showed the animal to be in poor general condition. He demonstrated tenderness in the abdominal area. A tentative diagnosis of nephritis was made.

It was noted that a urinary catheter could be inserted only 1 inch because of massive accumulation of triple phosphate crystals in the urethra; a saline flush yielded 15 ml. of urine. A radiograph of the abdominal region showed the right kidney to be twice normal size (abscessed kidney).

The animal was treated with antibiotics for 8 days.

	Aug. 30	Aug. 31	Sept. 3	Sept. 7
Erythrocytes	7,250,000	5,510,000	6,140,000	4,830,000
Hemoglobin	13.6	9.0	9.8	9.0
PCV	43.0	31.0	32.5	30.0
MCV	59.3	56.3	52.9	62.1
MCHC	31.6	29.0	30.1	30.0
Sedimentation Rate	34/7 = +27	60/24 = +36	60/21 = +39	62/26 = +36
Icterus index	2.0	2.0	2.0	2.0
Buffy coat	4.0	3.0	2.5	1.2
Leukocytes	100,500	72,500	51,000	16,850
Bands	23.0	7.6	0	2.0
Neutrophils	60.0	84.0	89.0	77.0
Lymphocytes	2.0	4.0	7.0	11.0
Monocytes	15.0	5.0	4.0	10.0
Culture: <i>Staphylococcus pyogenes</i>				
Non-protein nitrogen	35.0 mg. per cent.			
CO ² combining power	37 vol. per cent			

Urine*

	cloudy	very cloudy	slightly cloudy
Transparency	cloudy	very cloudy	slightly cloudy
Specific Gravity	1.031	1.028	1.026
pH	6.5	6.0	6.0
Protein	++	+++++	+
Bile	++++	negative	negative
Urobilinogen	++	trace	+
Occult Blood	+	+	negative
Casts	5 f.g./HDF	rare	rare
WBC	numerous	myriads	100/HDF
RBC	few	+++++	10/HDF

* f.g. — fine granular casts

HDF — high dry field of microscope.

Interpretation:

1. Hemoconcentration masked the anemia on first clinical examination. MCV is low and in this case suggests that erythrocyte counts are erroneously high. Anemia of infection is normocytic normochromic.
2. The high positive erythrocyte sedimentation rate indicates the existence of a significant tissue derangement.
3. A leukemoid reaction or *Regenerative Left Shift* is seen. The very high total WBC suggests localization of a bacterial infection with pus formation.
4. Relative lymphopenia, absolute monocytosis and absolute eosinopenia are present.
5. The high urinary protein level together with the findings in the radiograph suggest that the kidney may be introducing exudate into the glomerular filtrate.
6. The many granular casts on August 30th suggests a lesion of renal origin.
7. The bilirubinuria on August 30th indicates that hepatic regurgitation of bilirubin was occurring.

Case 3. Cellulitis in the Canine Associated with Anemia of Infection

A 7-year-old, female Golden Retriever was seen at the clinic on the 15th of May. The dog had a history of abscess in the lumbar region, persisting for seven weeks. The area had been surgically drained by the referring veterinarian.

Examination showed a thin, depressed animal in poor general condition with pale mucus membranes and a temperature of 103.1° F. There were multiple abscesses and fistulous tracts of the left lumbar region which extended down the left hind leg and up over the dorsum to the right lumbar region. Ten or twelve openings to the tracts were draining a thick white pus which contained yellowish granules. Bacteriologic culture showed *Staphylococcus pyogenes*.

The animal was treated systemically with penicillin and streptomycin; the abscesses were treated locally with peroxide flushes. On the seventh day of hospitalization, the patient died.

Blood studies were conducted at the intervals indicated:

	15 May	16 May	19 May
Erythrocytes	2,460,000	2,300,000	2,780,000
Hemoglobin	5.3	5.3	6.1
PCV	16.0	16.0	18.0
MCV	65.0	69.5	64.7
MCHC	33.0	33.0	33.8
Sedimentation rate	79/61 = +18	78/61 = +17	75/55 = +20
Icterus index	20.0	20.0	20.0
Buffy coat	3.5	4.0	5.0
Leukocytes	66,700	87,750	96,000
Metamyelocytes	1.0	2.0	1.0
Bands	15.0	19.0	15.0
Neutrophils	69.0	68.0	74.0
Lymphocytes	4.0	2.0	2.0
Monocytes	11.0	9.0	8.0

<i>Urinalysis</i>	<i>15 May</i>
Color	orange
Specific gravity	1.015
pH	5.5
Protein	+++
Acetone	negative
Sugar	present
Bile	+++
Urobilinogen	negative
Occult blood	negative
Sediment: Moderate number of coarsely granular casts, few RBC and epithelial cells. The van den Bergh test showed a positive direct reaction, serum bilirubin was 4.4 mg. % The BSP test showed 46% retention in 15 minutes, 34% in 30 minutes (indicates liver damage).	
Bone marrow aspiration (18 May) showed a M:E ratio of 12.7:1.	
A radiograph showed splenomegaly and soft discrete densities in the anterior lobes of the lungs.	
The pathologic diagnosis established at necropsy:	
(1) cellulitis.	
(2) mycotic pneumonia (branched fungus mycelia were seen in lung sections).	
(3) pancreatic atrophy.	

Interpretation:

1. Advanced normocytic normochromic anemia resulting from depression of erythropoiesis by chronic bacterial infection.
2. Marked leukocytosis is common to localized pus formation. Regenerative left shift with absolute monocytosis and absolute eosinopenia and relative lymphopenia. Stress reaction and chronicity reflected by this differential leukocyte count. The high and continuously increasing leukocytosis is a grave sign.
3. Icterus index of 20 suggests liver damage.
4. Erythrocyte sedimentation rate of +17 to +20 is to be considered a significantly elevated corrected rate in the face of the low PCV.

Case 4. Pyometra in the Canine

A 7-year-old, female Boxer on admission showed depression, some degree of dyspnea, distended abdomen, and a temperature of 102° F. The pulse was strong and regular with a rate of 144. A bloody coagulated discharge between the lips of the vulva was noted. Although the patient's abdomen was rigid and palpation was difficult, a mass could be defined.

A radiograph showed the uterine horns to be enlarged. The NPN was 144.0 mg.%. A culture of the vulvar discharge showed *E. coli* to be present.

Erythrocytes	6,900,000	Leukocytes	82,500
Hemoglobin	15.3	Myelocytes	1.0
PCV	45.5	Metamyelocytes	2.5
MCV	65.9	Bands	24.25
MCHC	33.6	Neutrophils	59.5
Icterus index	5 units	Lymphocytes	3.5
Reticulocytes	0.4%	Monocytes	9.0
Buffy coat	4.5	Eosinophils	0.25

Abdominal surgery was performed; the uterus was enlarged (2½ inches in diameter, body 5 inches long, horns 15 inches long) and filled with a creamy, red, foul-smelling pus.

Interpretation:

1. Extreme leukocytosis is common to pyometra when there is retention of the exudate. With drainage of the purulent exudate the total leukocyte count falls (See Table 50, p. 314).
2. Regenerative left shift is to be anticipated. Absolute monocytosis is a common finding. Relative lymphopenia and relative eosinopenia are observed.
3. Anemia may develop with the PCV in the 30–35 per cent range.
4. NPN may be above normal as a result of decomposition of the retained purulent exudate.
5. Erythrocyte sedimentation rate is variable in pyometra.

Case 5. Malignancy in the Equine

A 13-year-old, Quarter Horse stallion was presented at the Clinic on March 19. Four weeks previously he had been treated for bots. Two weeks later he had stepped on a nail and received treatment. For 1 week before admission he had been off-feed.

Clinical examination showed the animal to be depressed and to have a temperature of 102.5° F. The left hind foot showed a nail hole at the point of the frog with slight swelling of the fetlock. The foot was apparently quite painful but there was no evidence of spread of infection. Feces were dry and hard.

By the afternoon of the second day of hospitalization the patient's condition had markedly deteriorated. Mucus membranes and conjunctiva were pale and a slight icteric cast of the latter was noted. Temperature was 104.0° F., the heart rate was over 100 with a beat sufficiently strong to be visible over the entire body. The animal was reluctant to move and refused food and water. He died that night.

At necropsy the animal was found to have a retained testicle showing adenocarcinoma (Sertoli cell). The spleen weighed 67 pounds; half this mass was composed of normal tissue, the balance was metastatic adenocarcinoma. The pleural cavity was filled with "watery blood," and a careful search failed to reveal a ruptured blood vessel.

Complete blood counts were taken at admission and on the following afternoon:

	19 March	20 March
Erythrocytes	4,850,000	3,840,000
Hemoglobin	8.2 gm.	6.2 gm.
PCV	25.0	19.0
MCV	51.5	49.4
MCHC	32.8	32.6
Icterus index	15 units	50 units
Leukocytes	20,500	24,450
Bands	0.5	3.3
Neutrophils	90.0	91.3
Lymphocytes	7.5	3.7
Monocytes	2.0	1.7

Diagnosis: Adenocarcinoma of the testicle and spleen with anemia and terminal bleeding into the thoracic cavity.

Interpretation:

1. The anemia is normocytic normochromic.
2. Icterus index increasing from 15 to 50 units in a 24-hour period may reflect increased bilirubin production from free blood in the thoracic cavity. (However, it should be recalled that one literature report claims that the bilirubin concentration in equine plasma may more than double in a 48-hour fasting period.)
3. Stress phenomenon is apparent in the absolute neutrophilia associated with absolute lymphopenia and absolute eosinopenia.
4. Total leukocyte count of 20–24,000 is to be regarded as a significant elevation in the horse. Leukocytosis with neutrophilia is to be anticipated in a malignancy.
5. The extreme neutrophilia with absolute lymphopenia reflect a grave situation.

Case 6. Malignancy in the Canine

A 14-year-old, female Irish Setter was admitted on February 25. The animal had been treated for pleurisy and the referring veterinarian had diagnosed a "bad heart."

Auscultation revealed dullness over the left thorax and the heart sounds were weak. The temperature was 103° F. A tentative diagnosis of hydropericardium was made.

A radiograph showed considerable fluid to be present in the left thorax associated with a collapsed left diaphragmatic lobe and a thickened left pleura. Diagnosis: Primary pulmonary neoplasm.

Thoracentesis produced almost a liter of dark sanguineous fluid:

Specific gravity	1.018	
Erythrocytes	825,000/cmm.	
Leukocytes	2,800/cmm.	(Neutrophils, 59%; small mononuclear cells, 25%; and large mononuclear cells, 16%)
Cell rafts	680/cmm.	

The cell rafts showed rare mitoses, basophilia, variation in nuclear and cell size, crowding of cells in the mass, and vacuolation of the individual cells.

A pulmonary lobectomy was performed on February 28. Good recovery was made from the surgery and a week later thoracentesis yielded a dark sanguineous fluid:

Specific gravity	1.019	
Erythrocytes	2,280,000	
Leukocytes	11,600/cmm.	(Neutrophils, 64%; small mononuclears, 5%; and large mononuclears, 30% [highly vacuolated])

No rafts of cells were seen.

Blood examination at second thoracentesis:

Erythrocytes	8,020,000	Icterus index	hemolyzed
Hemoglobin	18.8	Leukocytes	41,900
PCV	56.0	Bands	1.7
MCV	69.8	Neutrophils	92.3
MCHC	33.6	Lymphocytes	1.3
Sedimentation rate	6/ no correction	Monocytes	4.7
Marked rouleau formation.			

The following day the animal was put to sleep. Necropsy showed excellent healing of the incision. Multiple small foci of carcinoma were found in the remaining lung and in the regional lymph nodes; the diagnosis was:

1. Carcinoma, bronchogenic with metastasis.
2. Subacute glomerulonephritis.

Interpretation:

1. Hemoconcentration is indicated by a PCV of 56 per cent and a hemoglobin of 18.8 gm. per cent. In view of the fact that malignancy leads to depression of erythropoiesis, the anticipated anemia is masked.
2. An erythrocyte sedimentation rate of 6 mm. in the face of a PCV of 56 is a highly significant result reflecting existence of advanced pathology.
3. The stress phenomenon is shown by the elevated total leukocyte count associated with absolute neutrophilia, lymphopenia and eosinopenia.
4. The extreme neutrophilia is an unfavorable sign.
5. Rafts of cells in the thoracic fluid point to the presence of neoplasia.

Case 7. Multiple Abscesses in the Equine with Associated Anemia of Chronic Infection

A 15-month-old, Thoroughbred mare had been on pasture and within the past week, the owner had noticed swellings over the animal's body.

Clinical examination showed an animal in poor condition with a temperature of 103.8° F. and pulse rate of 64. Growth had been stunted, hair coat was dry and the animal was emaciated. There were multiple fluctuating swellings over the body, some of these were abscessed. There was a pneumonia with consolidation in the ventral portions of the lungs.

A blood examination was made on the day of admission:

Erythrocytes	5,560,000	Leukocytes	31,800
Hemoglobin	6.5	Myelocytes	1.0
PCV	22.0	Metamyelocytes	4.0
MCV	39.5	Bands	5.5
MCHC	29.5	Neutrophils	80.0
Icterus index	5.0	Lymphocytes	7.0
Buffy coat	1.5	Monocytes	1.5
		Unclassified	1.0

A radiograph of the chest showed a hydrothorax, the presence of pleural adhesions and bronchopneumonia.

The following day the chest was tapped and a sanguineous fluid obtained:

Erythrocytes—169,000/cmm., Leukocytes—89,200/cmm., 95% neutrophils.

Bacterial culture: *Streptococcus equi*. Total protein—4.2 grams %.

Euthanasia was advised and at necropsy the findings, in addition to multiple subcutaneous abscesses, were several fractured ribs showing fibrous callus and a serofibrinous pleuritis and pericarditis.

Interpretation:

1. The anemia is of the depression type. The red cells are normocytic and slightly hypochromic.
2. The total leukocyte count is markedly elevated for the equine. Abscess formation stimulates leukocytosis.
3. There is a regenerative left shift with absolute lymphopenia and absolute eosinopenia. Stress reaction.
4. A marked absolute reduction in lymphocytes and extreme neutrophilia are unfavorable signs.
5. The fluid aspirated from the chest was exudative and of infectious origin as evidenced by the neutrophilia, protein content, and the isolation on culture of *Streptococcus equi*.

Case 8. Traumatic Reticulitis in the Bovine

The ambulatory crew was called to a local dairy to examine a mature Holstein cow that was "off-feed and off-milk."

When examined the cow had a temperature of 103.4° F., rumen contractions were 1 per minute and the heart rate was 68 per minute. A grunt was elicited on xyphoid pressure.

Erythrocytes	5,520,000	Leukocytes	23,650
Hemoglobin	9.9 grams	Bands	2.0
Packed Cell Volume	31.0	Neutrophils	86.5
MCV	56.0	Lymphocytes	8.5
MCHC	32.0	Monocytes	3.0
Icterus index	5 units		
Buffy coat	2.0		
Moderate rouleau formation was observed.			

The cow was put on penicillin-streptomycin medication. On the day following the examination a rumenotomy was performed. A penetrating wire was found in the left lateral wall of the reticulum. There were considerable adhesions in the area. Many other free wires were found in the reticulum. Antibiotic therapy was continued for 3 more days. The temperature returned to normal and rumen motility increased to 2 per minute. Recovery was complete and the stitches were removed on the seventeenth post-surgical day.

Interpretation: (Also, see Table 56, p. 327.)

1. The erythrocyte count and hemoglobin concentration are in the low normal range.
2. The total leukocyte count is very high for the bovine and reflects pus formation.
3. A strong response to stress is indicated by the leukocytosis, absolute neutrophilia, absolute lymphopenia and absolute eosinopenia. The monocyte distribution is normal.
4. Erythrocyte rouleau in the bovine indicates the existence of a serious disease state.

Case 9. Leptospirosis in the Canine

A 9-month-old, female Weimeraner was admitted to the clinic on May 10. The dog had been seen previously 1 month prior to admission and at that time it had a blood-diarrhea and it was vomiting. The tonsils were reddened but were not out of their crypts, a bilateral conjunctivitis was present. The animal was hospitalized for 10 days and discharged.

Clinical examination on second admission showed a temperature of 100.6° F. and the presence of mouth ulcers, vomiting, diarrhea and listlessness.

The animal was treated with streptomycin, her condition did not improve and she died on May 18.

	11 April	14 May	15 May
Erythrocytes	6,300,000	6,460,000	6,110,000
Hemoglobin	14.9	13.8	14.5
PCV	46.0	42.0	43.0
MCV	73.0	65.0	70.0
MCHC	32.4	32.8	33.7
Sedimentation rate	5/4 = +1	14/8 = +6	35/7 = +28
Icterus index	<5	<5	<5
Buffy coat	1.0	1.0	1.5
Leukocytes	13,950	25,750	38,700
Bands	0.0	2.7	1.7*
Neutrophils	67.5	77.3	83.3*
Lymphocytes	17.0	8.7	5.0
Monocytes	9.5	10.0	6.0
Eosinophils	6.0	0.3	0.0
Degenerated cells	0.0	1.0	4.0
		* toxic vacuolation	
NPN		337.5 mg%	375.0 mg%
Agglutination Test:	Leptospira canicola 1:1000		
	Leptospira icterohaemorrhagiae 1:100		

The pathologic diagnosis was leptospirosis and acute nephritis. Gross findings at necropsy included ulcers of the buccal mucosa, dark red stomach in which the mucosa was completely destroyed and replaced with fibrino-necrotic material. Liver and kidneys were swollen, bronchiolitis and peri-bronchiolitis were present. Histopathology showed intense fatty infiltration of the liver with atrophy of the hepatic cords, cortical areas of the kidney were infiltrated with monocytes and plasma cells, many leptospira were demonstrated in the convoluted tubules by Levaditi's stain.

Interpretation:

1. Red cell count and hemoglobin are normal. No hemolytic destruction as shown by normal icterus index.
2. Elevated erythrocyte sedimentation rate is to be anticipated. Neutrophilia with relative lymphopenia and absolute eosinopenia are a response to stress.
3. The toxic vacuolation of neutrophils is a grave sign.
4. The high non-protein nitrogen reflects acute nephritis leading to uremia.

Case 10. Chronic Interstitial Nephritis in the Canine

A 10-year-old, male Australian Shepherd dog was hospitalized in November for severe otitis externa. He was again presented in the following January with a history of alternate bouts of stiffness. He had recently shown anorexia,

polydipsia and polyuria. His oral mucous membranes were pale but not ulcerated and the breath had an uremic odor.

The patient showed marked depression and by the third day he was very weak; respirations were shallow and irregular. He was found dead in his cage the following morning. The pathologic diagnosis was chronic interstitial nephritis and cystitis.

		<i>Urine</i>	
Erythrocytes	3,670,000	Specific gravity	1.012 (low)
Hemoglobin	8.8	pH	5.0
PCV	26.0	Protein	two plus
MCV	70.8	Acetone	negative
MCHC	33.8	Sugar	three plus
Sedimentation rate	67/34 = +33	Indican	negative
Icterus index	<5	Bile	negative
Reticulocytes	0.6%	Urobilinogen	negative
Nucleated erythrocytes	0.0	Occult blood	negative
Slight anisocytosis and poikilocytosis		Chlorides	1 gm./liter (low)
Leukocytes	12,450	Sediment:	no casts were seen
Bands	1.0		3-5 WBC/HDF
Neutrophils	77.5		7-10 RBC/HDF*
Lymphocytes	7.0		
Monocytes	14.5		
NPN	270.0 mg. % (uremia)		
Calcium	7.0 mg %		
Phosphorus	35.5 mg % (very high retention)		
Alkaline phosphatase	2.0 units		
CO ₂ combining power	11.4 vol. % (marked acidosis)		

* High dry field.

Interpretation:

1. Advanced normocytic normochromic anemia as a result of selective depression of erythropoiesis. This is a common finding in chronic interstitial nephritis with uremia (Table 49, p. 313).
2. The plasma in the hematocrit is commonly colorless due to the low concentration of bilirubin.
3. An elevated erythrocyte sedimentation rate is to be anticipated in C.I.N.
4. Reticulocyte percentage is inadequate to overcome the anemia.
5. Monocytes are at upper normal absolute level. Increase in monocytes is not typical of C.I.N.
6. Absolute lymphopenia is typical especially in dogs over 5 years of age with C.I.N. Neutrophilia is common.
7. Renal failure is evident from the exceptionally high NPN and serum phosphorus level. CO₂ combining power is dangerously low and suggests a grave prognosis.
8. The low specific gravity and moderate proteinuria, as well as the lack of urine chlorides, are all typical of advanced nephritis with vomiting.

Case 11. Chronic Interstitial Nephritis with Uremia in the Canine

A 10-year-old, male Chow-German Shepherd cross had vomited twice the week before and again 3 days later. He had not eaten well since then and vomited again on the day of admission to the clinic. He had shown difficulty in walking and was observed to drink a lot of water.

Clinical examination showed a depressed animal with a temperature of 98.6° F., foul odor to breath, teeth full of tartar. His pulse was firm and

strong, the heart somewhat muffled. His abdomen was tense and sensitive; mucus membranes were pale. When first seen he exhibited clonic spasms of the face.

		<i>Urine</i>	
Erythrocytes	6,860,000	Specific gravity	1.018
Hemoglobin	13.8	pH	7.5
PCV	42.0	Protein	three plus
MCV	61.2	Sugar	negative
MCHC	32.8	Bile	negative
Sedimentation rate	41/8 = +33	Chlorides	1.0 gm. /liter (Low due to loss of chlorides through vomiting).
Icterus index	<5	Sediment:	No casts were seen; rare RBC and WBC, large numbers of triple phosphates were present.
Reticulocytes	0.1%		
Nucleated erythrocytes	none seen		
Slight anisocytosis and poikilocytosis			
Leukocytes	14,400		
Bands	1.5		
Neutrophils	93.5		
Lymphocytes	2.5		
Monocytes	2.0		
Unclassified cells	0.5		
Non-Protein Nitrogen	498.0 mg. per cent (uremia).		

The animal died on the day following admission. The pathologic diagnosis was nephritis, chronic interstitial. Both kidneys were shrunken, nodular, and pale in color. Histologically they exhibited marked interstitial fibrous and parenchymal atrophy. The liver showed widespread congestion, there was slight enlargement of the parathyroid glands, the intercostal pleura presented areas of calcification. No calcification was seen in the cardiovascular system.

Interpretation:

1. Presence of anemia may be masked by hemoconcentration. If anemia exists, it is bordering upon microcytic normochromic.
2. High erythrocyte sedimentation rate is a common finding in chronic interstitial nephritis.
3. The low percentage of reticulocytes suggests depression of erythropoiesis which in time would lead to anemia of nephritis.
4. The total leukocyte count generally remains in the upper normal range but absolute neutrophilia and absolute lymphopenia are common findings, especially in older dogs with chronic interstitial nephritis.
5. In some instances the eosinophil count is elevated but in this case there is absolute eosinopenia. Thus the eosinophil picture in C.I.N. is variable. Monocytes generally are not involved.
6. Low specific gravity, proteinuria and chloride retention by the kidney are all suggestive of advanced chronic renal disease.
7. The absence of casts is due to the alkaline urine (in which they dissolve). The alkaline urine most likely represents advanced tubular deficiency and loss of the ability to acidify the urine by active ion transport.
8. NPN level of 498 mg. per cent is a grave sign and suggests the terminal nature of the case.

Case 12. Hypothyroidism in the Canine

A 3-year-old, male French Poodle was presented on November 16. The disease had been diagnosed by the referring veterinarian as an allergic derma-

titis which had improved temporarily with steroids and topical medication.

The animal had had heavy dandruff for a year and a half and exhibited solidification of oil ducts over most of the body. The dog was heavy, listless, and not willing to play.

Clinical examination showed diffuse areas of alopecia over the ribs, abdomen, and inner thighs. There were small focal areas of proliferated sebaceous material over the whole body, most noticeable in the neck area and ear margins. The pores over the abdomen were large and filled with waxy material. The skin was thick and had a rubbery texture. The blood cholesterol was 428 mg. per cent.

On November 20, biopsy material was taken surgically from the thyroid and skin areas: The thyroid specimen was predominantly parathyroid; there was a small amount of atrophic thyroid gland with collapsed follicles, a few contained colloid. The biopsy of the skin revealed atrophic glands and hypokeratinization of the epidermis.

A protein bound iodine value of 2.98 micrograms per 100 ml. of serum was determined on November 26 and thyroid therapy was instituted on December 1. The dog was discharged and returned as an outpatient a month later for a progress check. A complete personality change had occurred, he was more alert and active. His skin was more elastic and pliable but some dermatitis persisted. The blood cholesterol was 176 mg. per cent. He was seen again in March and showed much improvement. Thyroid therapy was continued.

	16 November	27 November	12 December	11 January
Erythrocytes	4,390,000	4,410,000	4,770,000	5,480,000
Hemoglobin	10.6	10.6	12.1	13.8
PCV	31.5	32.0	36.5	41.0
MCV	71.7	72.5	76.5	74.8
MCHC	33.6	33.1	33.1	33.6
Sedimentation rate*	18/23 = -5	12/22 = -10	10/14 = -4	1/9 = -8
Icterus index	>5	>5	7.5	2.0
Reticulocytes	0.1%	—	1.6%	0.1%
Hypochromasia	slight	slight		
Target cells	frequent	occasional	occasional	frequent
Leukocytes	11,850	10,700	14,450	12,300
Bands	2.5	2.0	0.5	1.5
Neutrophils	69.5	65.0	82.0	67.5
Lymphocytes	18.5	25.5	14.5	19.0
Monocytes	6.5	6.5	2.5	6.5
Eosinophils	2.5	0.5	0.5	5.5
Unclassified cells	0.5	0.5	0.0	0.0

* Erythrocytes scattered throughout plasma; no clear plasma at top followed by hazy red plasma as in a true diphasic reaction. Diffuse sedimentation rate associated with erythrocytes of abnormal morphology (leptocytes).

Interpretation:

1. Hypothyroidism is often associated with slight to moderate normocytic normochronic anemia. Thyroxin is one of the hormones that influences erythropoiesis.
2. The negative corrected erythrocyte sedimentation rate is associated with the occurrence of leptocytes.
3. The findings of elevated cholesterol (>250 mg.%) and low PBI (<3 μ g.%) suggest thyroid insufficiency. A more certain test would be the study of the uptake of iodine¹³¹.

Case 13. Blood Loss Anemia in the Equine

An 8-year-old Saddlebred gelding was admitted on September 12 for surgery. The animal had been injured about 18 months previously. At that time his neck became swollen and subsequently developed a raw area. A diagnosis of equine sarcoid had been established by biopsy.

Physical examination showed a subepithelial mass on the right side of the neck 12 cm. in diameter; most of the tumor was covered by skin, one small area protruded.

Surgery was performed on September 13. The horse lost a large amount of blood during surgery and the wound continued to ooze blood for several days following the operation. A complete blood count was performed on the seventh post-surgical day and at frequent intervals for the next 2 weeks. The animal developed a moderately elevated temperature between the eighth and fourteenth days; during this period the wound exhibited considerable necrotic tissue. Penicillin therapy was instituted.

The animal was hospitalized until the wound was entirely healed over, a period of 5 months. Although the prognosis at discharge was "poor," information was received 18 months later that the horse was being entered in shows.

	<i>Day 7</i>	<i>Day 8</i>	<i>Day 9</i>	<i>Day 10</i>	<i>Day 12</i>	<i>Day 13</i>	<i>Day 14</i>	<i>Day 16</i>	<i>Day 20</i>
Erythrocytes	2.42	2.71	2.35	2.94	2.41	2.88	3.93	3.03	4.50
Hemoglobin	4.5	5.0	4.4	6.2	4.2	5.9	7.2	5.3	7.7
PCV	12.5	15.5	13.0	18.0	14.0	15.5	23.0	17.0	25.0
MCV	51.6	57.2	55.3	61.2	58.1	53.8	58.5	56.1	55.5
MCHC	36.0	32.2	33.8	34.4	30.0	32.9	31.3	31.2	30.8
Icterus index	5	5	5	3	5	7	7	5	5
Reticulocytes	0.6	0.0	0.1	1.0	0.1	0.1	0.0	0.0	0.1
Nucleated erythrocytes	0	0	0	4.5	0	0	0	0	0
Leukocytes	13.5	18.8	19.5	16.6	17.3	21.8	28.0	29.5	19.4
Metamyelocytes	0	0	1.0	0	0.5	0	0	0	0
Bands	2.5	3.5	2.0	0	3.0	7.0	10.5	3.5	1.0
Neutrophils	84.5	79.5	86.0	81.5	86.0	82.5	77.5	84.0	76.5
Lymphocytes	9.0	9.5	6.5	8.0	5.0	8.0	7.0	9.0	19.5
Monocytes	3.0	5.0	3.0	8.0	4.0	1.5	4.5	3.0	2.5
Eosinophils	0.5	1.5	1.5	1.5	1.0	1.0	0.5	0.5	0.5
Basophils	0.5	1.0	0	1.0	0.5	0	0	0	0

Interpretation:

1. The patient was actively producing erythrocytes as indicated by the doubling of the PCV in 13 days.
2. Spectacular reticulocytosis was not observed although the released red cells were larger than normal as shown by the above normal MCV. There was also an absence of significant polychromatophilia. Under similar conditions of blood loss, other domestic species would have a significant reticulocytosis. Thus, the peripheral blood picture in the equine in response to hemorrhage differs significantly from the pattern of polychromatophilia and reticulocytosis as seen in other domestic animals.
3. The leukocytosis, absolute neutrophilia and slight to moderate left shift are to be anticipated in recovery from acute blood loss.

Case 14. Hemolytic Crisis in the Canine

The patient was a 5-year-old, male collie dog. The only history was for the preceding 2 weeks; at the beginning of this period the dog had a sudden

onset of paralysis of the hind quarters. No trauma was involved. The animal was able to eat, urinate and defecate; he could support his weight on his front legs but was unable to get up. During the first week a slight fever was noted.

The dog was admitted to the clinic on November 15 with a temperature of 101° F., pulse rate of 60 and in generally good condition. He showed no extensor reflexes in front or back, no placing reflexes. His plantar reflex was good in the left hind leg, but was not consistent in other legs. Deep pain perceptrors were functional in all four legs.

Because of an extensive skin irritation, the animal was clipped, washed and dried and pellitol ointment was applied over the whole body area; this treatment was continued from November 18 through the 20th. On November 20, the animal developed hematuria and on December 2 the patient was in severe hemolytic crisis with pronounced hemoglobinemia and hemoglobinuria. The animal at this time was very depressed; 250 ml. of blood was given, the animal improved. Improved use of the limbs was evident by November 30. Hematuria appeared again December 25, urine was clear December 26, December 27 hematuria again. The dog was discharged on January 12 with no history of hematuria during the period January 7 to January 12.

A NPN was run on November 19: 45.5 mg. per cent. An erythrocyte fragility test on December 6 was normal (initial hemolysis 0.46% saline). Urine on December 2, December 6 and December 7 showed "myriads" of erythrocytes in the sediment.

	19 Nov.	2 Dec.	6 Dec.	7 Dec.
Erythrocytes	7.77	2.16	3.21	3.22
Hemoglobin	15.4	7.0	8.6	9.0
PCV	49.0	18.0	26.0	28.0
MCV	63.1	83.3	80.9	86.9
MCHC	31.4	38.8	33.1	32.1
Sedimentation rate	7/1 = +6	20/55 = -35	61/34 = +27	—
Nucleated erythrocytes	0	26.0	53.0	37.0
Polychromatophilia	none	moderate	marked	marked
Leukocytes	8.65	32.25/29.9	40.10/31.8	25.25/19.7
Metamyelocytes	0	1.0	1.0	1.0
Bands	2.0	8.0	14.0	18.0
Neutrophils	67.0	73.0	52.0	61.0
Lymphocytes	14.0	5.0	15.0	8.0
Monocytes	16.0	10.0	16.0	11.0
Eosinophils	1.0	3.0	2.0	1.0
Body Temperature	101.4	104.0	102.6	102.8

The dog was not seen in this clinic again. Word was received that the animal died during another attack of paralysis and hematuria.

Interpretation:

1. Very active erythrogenesis is reflected in the increase in MCV from 63 to 87 cubic microns.
2. The negative corrected erythrocyte sedimentation rate on December 2 reflects a high level of immature red cells. The positive ESR four days later may be in part related to the blood transfusion.
3. The elevated temperature was to be anticipated with massive hemoglobin breakdown.
4. MCHC of 39 on December 2 is high and is due to free hemoglobin in the blood plasma.

5. Leukocytosis with left shift is to be anticipated in hemolytic crisis.
6. The great number of nucleated erythrocytes in peripheral blood is the result of intense erythrogenesis in response to the anemia.

Case 15. Chronic Blood Loss in the Canine

A 2-year-old, female Rat Terrier was presented on September 24 with a history of anorexia and depression.

Clinical examination showed a weak, dehydrated animal in poor condition and exhibiting very pale mucus membranes. The temperature was 99.8° F. The animal was covered with stick-tight fleas (*Echidnophaga gallinacea*).

The fleas were removed, 80 ml. of whole blood was administered intravenously and an oral hematinic (Iberol) was prescribed. The animal improved and was discharged on October 2.

	24 September	30 September
Erythrocytes	590,000	3,120,000
Hemoglobin	1.6	6.8
PCV	5.0	23.0
MCV	84.7	73.7
MCHC	32.0	29.5
Sedimentation rate	95/95 = 0	Diphasic, unreadable
Icterus index	<5	2 (opalescent plasma)
Reticulocytes	0.8%	6.2%
Nucleated erythrocytes	1.5	2.0
Anisocytosis	—	Moderate
Hypochromasia	Moderate	—
Polychromatophilia	Moderate	Marked
Target cells	Moderate	Moderate
Buffy coat	2.0	3.0
Leukocytes	13,600	12,300
Bands	1.0	0.5
Neutrophils	82.0	48.0
Lymphocytes	15.5	28.5
Monocytes	1.5	14.0
Eosinophils	0.0	9.0

This case demonstrates the potential of the hematopoietic system for replacing red cell volume when blood loss is stopped and erythrocyte production is in high gear.

Interpretation:

1. This is an extreme case of anemia as indicated by a PCV of 5 per cent, hemoglobin of 1.6 gm., and erythrocytes of less than one million per cmm. In slowly developing anemia, the vascular system makes certain adjustments to more efficiently use the reduced number of erythrocytes, *e.g.*, hypertrophy of the heart, increased heart rate, constriction of the vascular beds in all but vital organs, reduction in plasma proteins to reduce blood viscosity, and increased depth of respiration.
2. Following therapy, the diphasic erythrocyte sedimentation rate indicates active erythrogenesis. This is also shown by reticulocytosis.
3. The MCV of 85 $c\mu$ on September 24 is perhaps incorrect and the result of an inherent error in making an accurate red cell count in the face of low numbers of erythrocytes.
4. Reasons for a leukocyte picture as seen on September 30 are not apparent. With active erythrogenesis one generally anticipates a leukocytosis with neutrophilia and a left shift.

Case 16. Megaloblastic Anemia in the Canine (See page 351)

A 2-year-old, male Beagle was admitted on April 9. On February 6 of the same year the dog had shown convulsions. On admission the dog had a temperature of 102.5° F. His hair coat was thin, rough and dry. Mucosae were very pale. Small muscle groups in the shoulder and thigh areas exhibited spasmodic twitching. Blood transfusions were given: 250 ml., April 9 and 10; 150 ml., April 23; 250 ml., April 28. A hematinic was given daily by mouth from April 15 through May 9 after which no further treatment was given and the patient was released July 14.

A radiograph of the abdomen on April 10 revealed a greatly enlarged spleen. A second radiograph taken May 1 showed the organ to be "not quite so large."

The blood cholesterol was 309 mg.% and the NPN 24 mg.% on April 14. The urine on April 9 showed a trace of albumin, trace of bile, 4 plus urobilinogen, and chlorides 1.5 Gm./liter. There were a few finely granular casts, and small numbers of RBC and WBC.

Interpretation:

1. This is a case of "Idiopathic" anemia for the cause was undetermined.
2. It was not possible to determine from peripheral blood the nature of the anemia. Erythrocyte morphology indicated a normocytic normochromic anemia and suggested selective depression of erythrogenesis. The high MCHC on admission suggests the possibility of some free hemoglobin in the plasma.
3. The blood cholesterol of 309 mg. per cent is just above the normal range and not quite high enough to suggest hypothyroidism. The skin and hair coat were not indicative of a state of hypothyroidism.
4. A non-protein nitrogen determination, as well as urine studies, excluded nephritis as a cause of the anemia.
5. The patient did not show signs suggesting an anemia due to chronic infection.
6. The diagnosis remained open so a bone marrow aspiration was made. This revealed an abnormally high number of rubriblasts and prorubricytes indicating maturation arrest. Normally with this type of maturation arrest the anemia should be macrocytic and Vitamin B₁₂ and folic acid are indicated.
7. The remission experienced in this case may have been due to the blood transfusions which reduced demands on the bone marrow for erythrogenesis and thereby gave the red cell maturation series a rest and possibly the opportunity to adjust to a more normal maturation in the presence of the essentials for red cell production provided by the hematinic. A maturation arrest in the early precursors of the red cell series points to a need for vitamins B₁₂ and folic acid.
8. In order to determine whether there would be a continuing need for the hematinic, the patient was taken off therapy and observed for 2 months. No relapse was experienced and therefore the case was discharged without prescribing continuation of hematinic therapy.
9. This is one of the few cases of anemia in the canine where a hematinic was justified. The secondary anemias associated with nephritis, chronic infection, malignancy, or hypothyroidism do not respond to hematinic preparations. The anemia in these cases is not the result of a lack of vitamins or iron but rather is the result of a toxic depression of erythrogenesis.

Case 16. Megaloblastic Anemia in the Canine

	April 9	10	14	21	28	May 2	7	22	June 2	July 7
Erythrocytes	1.40	2.16	1.78(?)	1.48	0.85	2.10	4.06	5.10	5.50	5.62
Hemoglobin	3.5	4.9	5.3	3.6	2.1	5.4	9.9	11.8	12.6	12.5
PCV	9.0	14.0	16.0	10.0	6.0	19.0	34.0	38.0	41.0	39.0
MCV	64.3	64.8	89.8(?)	67.5	70.5	90.5	83.7	74.5	74.5	69.4
MCHC	38.8	35.0	33.1	36.0	35.0	28.4	29.1	31.0	30.7	32.0
Sedimentation rate	87/+5	80/+13	77/+16	42/-37	93/-2	20/*-32	10/*-8	3/-9	2/-7	3/-8
Reticulocytes	0.1	0.1	—	0.1	8.1	11.4	9.3	0.3	0.7	0.5
Nucleated erythrocytes	0	0	0	1	2.5	13.0	7.0	0	0	1.0
Anisocytosis	slight	slight	slight	slight	marked	moderate	moderate	slight	slight	slight
Poikilocytosis	slight	slight	slight	slight	marked	moderate	moderate	slight	slight	slight
Hypochromasia	slight	slight	slight	slight	marked	moderate	moderate	slight	slight	slight
Icterus index was less than 5 units in all determinations										
Leukocytes	19.7	12.8	9.2	6.0	20.75	16.9	13.80	20.8	9.5	11.9
Myelocytes	0	0	0	0	1.0	0.5	0	0	0	0
Metamyelocytes	0	0	0	0	2.0	1.0	0	0	0	0
Bands	5.5	8.0	2.0	2.0	9.5	3.0	0	0	3.0	0
Neutrophils	71.0	72.5	65.0	65.0	60.5	59.0	62.5	82.5	69.5	65.0
Lymphocytes	16.5	15.0	19.0	22.0	15.0	18.0	26.5	9.5	21.0	25.0
Monocytes	7.0	4.5	13.0	11.0	12.0	16.5	10.0	6.0	4.5	7.0
Eosinophils	0	0	1.0	0	0	2.0	1.0	2.0	2.0	3.0
Bone marrow aspiration on:										
Rubriblast	10 April	6.4 (megaloblasts)			7 May				28 May	
Prorubricyte	16.2				1.3				0.3	
Rubricyte	10.1				2.7				2.2	
Metarubricyte	4.0				17.0				13.7	
Blast	1.4				26.7 (remission)				19.7	
Progranulocyte	1.7				0				0	
Myelocytes (neutrophilic)	5.0				2.2				1.3	
Myelocytes (eosinophilic)	0				8.4				10.9	
Metamyelocytes (neutrophilic)	9.9				0				1.4	
Bands (neutrophilic)	12.4				9.2				10.9	
Bands (eosinophilic)	0.7				12.7				14.0	
Neutrophils	18.7				0.7				0.3	
Eosinophils	0				16.3				16.5	
M:E	1.33:1				0				0.5	
Miscellaneous cells	15.6				1.03:1				1.55:1	
					2.8				8.3	

* Diphasic erythrocyte sedimentation

Case 17. The Hematology of Anaplasmosis (See page 353 for an interpretation of these data)

	17th	21st	25th	26th	27th	30th	31st	32nd	33rd	35th	37th	39th	45th	66th
Erythrocytes	—	—	7,95	6.55	6.15	3.16	2.58	2.12	1.67	1.24	1.11	1.41	2.42	4.88
Hemoglobin	12.0	11.4	13.0	10.7	9.7	4.4	3.8	3.4	3.4	3.4	3.4	3.8	5.0	7.9
PCV	34.0	32.5	36.5	30.5	28.0	13.0	12.0	11.0	12.0	12.0	13.0	14.0	18.5	24.0
MCV	—	—	50.3	46.6	45.5	41.1	46.5	51.8	71.8	96.7	117.1	99.3	76.4	49.2
MCHC	35.3	35.1	35.6	35.1	34.6	33.8	31.7	30.9	28.3	28.3	26.1	27.1	27.0	32.9
RBC	—	—	—	—	30% AB	—	50% AB	—	Many polychromatophilic macrocytes	—	—	Basophilic stippling	—	—
Morphology*	—	—	8,000	6,900	7,500	9,600	11,000	16,550	31,829	—	—	—	11,000	10,300
Leukocytes	Normal	N	N	N	N	Marked depression, anorexia, constipation and icterus	Down	Down	emaciated, labored breathing.	—	—	Up, eating a little.	—	—
Physical condition	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Temperature	101.8	101.8	101.8	101.2	101.0	104.9	101.8	100.4	92.0	100.0	—	101.6	102.2	101.8
Pulse	80	84	80	76	84	132	144	152	120	100	—	96	88	80
Respiration	Normal	N	N	N	N	56	N	16	20	20	—	20	20	44

* AB = *Anaplasma marginale* bodies.

Case 17. The Hematology of Anaplasmosis

A Guernsey heifer was given 10 ml. of blood by subcutaneous route from a cow that was a carrier of *Anaplasma marginale*. Blood drawn on the 10th and 12th days post-inoculation had a PCV of 31 and 32, respectively, and it was also normal in all other respects (see tabulated data on page 352). Compare the PCV, RBC, and MCV on 30th and 37th days post-inoculation. Note that the PCV is 13 per cent on each day but the erythrocyte number became reduced by two-thirds in that 7-day period. The size of the red cell had almost tripled. While there had been continuing destruction of the infected red cells, the bone marrow had been releasing immature erythrocytes of large size. Thus, a good bone marrow response was shown and prognosis was favorable. The leukocytosis was consistent with an anticipated increase in granulopoiesis in hemolytic crisis or acute blood loss. Anaplasmosis in the recovery phase is a transitory macrocytic hypochromic anemia. The body temperature of 104.9° F. on the 30th day was probably the result of rapid release of products of hemoglobin breakdown. A temperature rise is to be expected when large quantities of free hemoglobin or breakdown products of hemoglobin develop suddenly. This is an excellent example of how the bone marrow responds to massive blood loss or erythrocyte destruction. The greatest drop in PCV probably occurred on the 29th day and it was four days later that the first real evidence of a significant release of young erythrocytes to peripheral blood was seen. This is indicated by the rise in MCV and the presence of polychromatophilic macrocytes in large numbers. Although the red cell number continued to fall the mass of circulating red cells (PCV) became stabilized by the larger size of the erythrocytes. The crisis was met 10–11 days after erythrocyte destruction started (37th day) after which red cell number increased and red cell size decreased.

Case 18. Haemonchosis in the Ovine

A 1-year-old, male suffolk sheep had been showing edema of head and throat for 2 days in the month of October. One of several similar cases occurring over the past few years in this flock. The owner treated this animal

Erythrocytes	3.3	
Hemoglobin	2.9	
PCV	11.5	
MCV	34.7	
MCHC	25.2	
Icterus index	<5	
Reticulocyte count	5.7%	
Nucleated erythrocytes	1/100 WBC	
Anisocytosis:	Moderate to marked	Basophilic stippling: marked
Poikilocytosis:	" " "	Polychromasia: moderate to marked
Hypochromasia:	" " "	H-J bodies: occasional
Leukocytes	7,100	
Bands	1.0	
Neutrophils	38.0	
Lymphocytes	55.0	
Monocytes	2.5	
Eosinophils	3.0	
Degenerated cells	0.5	
Fecal Examination:	McMaster count—84,000 eggs per gram most of which were <i>Haemonchus contortus</i> .	

the day before with penicillin and phenothiazine (2 ounce drench). On physical examination the following were observed: temperature 102.5° F., pulse 136, respiration 28, mucous membranes pale, pupils dilated, moderate degree of nasal exudate, and marked depression.

Interpretation:

1. This is a blood loss disease which may be chronic or acute.
2. The anemia in this case is normocytic hypochromic.
3. The bone marrow is showing considerable evidence of active erythro-genesis but occurrence of poikilocytosis suggests that the released erythro-cytes are not capable of withstanding wear and tear of circulation. Thus, the blood loss is not compensated.
4. Basophilic stippling of erythrocytes is a common finding in both sheep and cattle in hemolytic crisis or blood loss diseases.

Case 19. Trichostrongylidosis in the Bovine

A Hereford steer, 1½ years old, representing one of a pair on dry pasture was showing an edematous swelling under the jaw which was first noticed 3 weeks ago. The other steer was not involved but both animals had been treated with phenothiazine by the owner when he first noticed the edematous swelling. The general condition of the patient was poor and its body temperature was 102.4° F.

Erythrocytes	2.5	Plasma Protein Analysis	
Hemoglobin	3.7	Total protein	2.8 gm.%
PCV	14.0	Albumin	1.8 gm.%
MCV	56.2	Globulin	1.0 gm.%
MCHC	26.4	A/G ratio	1.8
Icterus index not recorded, probably low.		Alpha globulin	0.3 gm.%
Anisocytosis:	moderate	Beta globulin	0.6 gm.%
Hypochromasia:	moderate	Gamma globulin	0.1 gm.%
Buffy coat	1.2	Fecal examination:	
Leukocytes	15,500	Flotation:	Trichostrongyles 5/L.P.F.
Bands	1.0	Stoll count:	Trichostrongyles 500/gm.
Neutrophils	50.0		
Lymphocytes	40.0		
Monocytes	8.0		
Eosinophils	1.0		

Interpretation:

1. Trichostrongylidosis in the bovine may produce a depression type anemia of advanced degree. In this case the anemia is normocytic and bordering on being hypochromic.
2. In this disease the leukocyte count is generally within the normal range and without significant changes in the differential count. When the leukocyte picture is altered, it is in the direction shown by this case: leukocytosis with moderate neutrophilia.
3. The edema of the throat is associated with a fall in plasma proteins as shown here.

4. The hypoproteinemia is of an advanced nature and indicates the chronic nature of this clinical condition. Serum protein levels should range between 5-8 Gm. per cent. Low colloidal osmotic pressure of the blood is responsible in part for the edema as observed in this case.

Case 20. Equine Infectious Anemia

An 8-year-old, male Quarter Horse was presented with the history of having a temperature of 105 to 106° F. for a period of 5 days in the fourth week of November. The patient had received 18,000,000 units of penicillin and a total of 2,500 grains of triple-sulfa. The clinical signs exhibited at that time were slight icterus, weakness, and poor appetite. Improvement occurred within 5 days. The animal became sick again in February and it was presented to the clinic on February 19. At that time, the clinical findings were as follows: body temperature 106.8° F., mucous membranes congested and icteric, abdomen tucked-up and sensitive to palpation, gait wobbly and shuffling behind, but the general condition was good.

	<i>Feb. 24</i>	<i>March 1</i>	<i>April 13</i>	<i>April 16</i>	<i>April 21</i>
Erythrocytes	9.10	8.54	5.90	5.64	3.8
Hemoglobin	12.25	10.9	7.13	8.3	6.09
PCV	36.0	36.0	23.0	24.0	17.0
MCV	39.5	42.1	38.9	42.5	44.7
MCHC	34.0	30.3	31.0	34.6	35.8
Sedimentation rate 3mm/10 min.		not done	55mm/10 min.	not recorded	66mm/10 min.
16mm/20 min.		not done	69mm/20 min.	66mm/20 min.	76mm/20 min.
Icterus index	20	100	100	20	50
Erythrocyte morphology	essentially normal, no young forms.				
Leukocytes	6,100	5,800	1,950	4,950	4,550
Band	0.0	0.0	3.0	0.0	0.0
Neutrophils	40.0	58.0	52.0	37.0	35.0
Lymphocytes	39.0	38.0	39.0	39.0	52.0
Monocytes	16.0	4.0	6.0	20.0	13.0
Eosinophils	5.0	0.0	0.0	2.0	0.0
Degenerated cells	0.0	0.0	0.0	1.0	0.0
Unclassified cells	0.0	0.0	0.0	1.0	0.0

Temperature readings as follows:

<i>February</i>	<i>March</i>	<i>March</i>	<i>April</i>
20th (AM) 107.0	1st (AM) 101.8	10th-16th 99.4-100.0	1st-3rd 99.0-100.0
(PM) 105.2	(PM) 99.6	17th-25th no record	4th 103.3
21st (AM) 102.5	2nd (AM) 101.6	26th 104.0	5th 106.7
(PM) 104.4	(PM) 101.2	27th 106.3	6th-11th 99.2- 99.6
23rd to 27th	3rd-6th	28th 104.3	12th 103.5
99.6-101.2	99.0-99.4	29th 100.0	13th 107.0
28th (AM) 106.8	8th 103.6	30th 99.2	14th-19th 99.6-100.8
(PM) 107.3	9th (AM) 103.0	31st 99.0	20th 106.8
	(PM) 101.6		21st 101.2
			22nd 104.4

Record of April 12: There has been a gradual decline in condition; horse now quite thin. Swelling of the scrotum was noticed today. This swelling was firm in nature.

April 20: Patient very weak, scrotum greatly enlarged and edematous; also, there is edema around muzzle and the mucous membranes are icteric.

April 22: Euthanasia and necropsy.

Interpretation:

- 1. Normocytic normochromic anemia produced by repeated hemolytic attacks. Note changes in icterus index.
- 2. Temperature rises were associated with red cell destruction.
- 3. Viral nature of the disease is reflected in the leukopenia.
- 4. Reduction of plasma proteins led to edema of muzzle and scrotum.

Case 21. Symptomatic Acquired Hemolytic Anemia in the Canine

The patient is a male English Springer Spaniel weighing 50 pounds. He became depressed on October 27 and was unable to get up. Body temperature was 104° F. The attending veterinarian administered penicillin and cortisone. The following day the patient was able to stand but he was weak; the body temperature was 102° F. The appetite increased and the dog became more active but on November 4, blood clots appeared in the feces. At this time the body temperature was normal and a fecal examination was negative for parasitism. On November 9, the patient was listless and the mucous membranes were pale. The attending veterinarian gave vitamin B₁₂, liver injections, and recommended a high protein diet. No improvement followed for the patient became weaker, slept a great deal, and acted as if not house broken. The patient was admitted on December 1 to the clinic of the School of Veterinary Medicine. The clinical record at the time of admission states that the patient was weak, listless, and had very pale mucous membranes. The body temperatures was 101.8° F.; the heart beat was strong and rapid and revealed a slight murmur.

A blood transfusion of 250 ml. was administered immediately and followed in 24 hours by a second transfusion of 275 ml. The patient was placed on KD ration and the hematinic, Iberol, was administered daily until January 9 when he was permitted to return home.

Laboratory studies at the School of Veterinary Medicine: fecal examination negative for parasites; non-protein nitrogen, 52.5 mg. per cent; urine analysis revealed evidence of kidney damage for there were many fine granular casts, white blood cells 15/high dry field and erythrocytes 10/HDF.

Bone marrow aspiration, iliac crest on December 3rd.

Blasts	1.3	Blasts	1.5
Progranulocytes	1.7	Prorubricytes	3.7
Myelocytes (neutrophilic)	2.4	Rubricytes	18.4
Myelocytes (eosinophilic)	0.3	Metarubricytes	38.4
Metamyelocytes (neutrophilic)	4.7		
Metamyelocytes (eosinophilic)	0.7	13% of metarubricytes showed <i>punctate basophilia</i> of cytoplasm (not normal)	
Bands (neutrophilic)	9.5		
Neutrophils	17.4	December 20—one RBC with punctate basophilia seen in blood film	
M:E = 38/62 = 0.61:1.0			

Symptomatic Acquired Hemolytic Anemia in the Canine

	Dec. 1	Dec. 3	Dec. 4	Dec. 11	Dec. 14	Dec. 20	Dec. 27	Jan. 2	Jan. 7
Erythrocytes	0.82	2.55	2.41	1.95	2.23	2.48	3.01	5.02	5.0
Hemoglobin	2.8	6.0	6.3	5.2	5.6	6.5	7.5	11.5	11.2
PCV	7.0	18.5	18.0	16.0	17.5	20.0	24.0	36.5	37.0
MCV	85.4	72.5	74.6	82.0	78.5	80.0	79.7	72.7	74.0
MCHC	40.0†	32.3	35.0	32.5	32.0	32.5	31.2	31.5	30.2
Sedimentation rate	87.0	70/+17	71/+16	71/+10	75/+19	69/+20	64/+26	15/+1	20/+7
Icterus index	10.0*	7.5*	7.5*	5.0	2.0	2.0	2.0	5.0	2.0
Reticulocytes	0.3	1.0	0.9	0.1	4.5	5.7	2.7	0.0	1.3
Nucleated erythrocytes	0	3.0	3.0	0.5	13.5	5.0	5.5	2.0	0
Spherocytes	+++								
Anisocytosis	-	+++	++	++	++	++	+	++	+
Leptocytes	++	++	++	++	++	++	++	++	+
Poikilocytosis	+	-	-	+	+	-	-	+	-
Polychromatophilia	++	+	+	+	++	++	++	+	+
Ghost erythrocytes	++	+	+	+	++	++	++	+	+
Clumped erythrocytes	++	+	+	+	++	++	++	+	+
Leukocytes	1.5	1.5	2.0	0.5	+	++	++	-	-
Buffy coat	15.7	15.1	17.1	6.5	1.5 red	1.0	0.5	0.5	1.0
Bands	2.0	1.0	3.0	2.5	13.0	11.45	11.5	11.7	9.2
Neutrophils	75.0	76.0	83.5	55.0	5.5	1.5	1.5	2.0	1.5
Lymphocytes	18.0	8.5	4.5	27.5	64.5	74.0	62.5	51.0	47.5
Monocytes	5.0	9.5	8.5	11.5	13.5	11.0	18.5	28.5	29.0
Eosinophils	0	4.5	0.5	2.0	15.0	10.0	15.0	15.5	11.0
Unclassified cells	0	0.5	0	1.5	1.5	3.5	2.5	2.0	9.5
Degenerated cells	0	0	0	0	0	0	0	0	1.5
									0

* Free hemoglobin in plasma above buffy coat.

† High MCHC related to free hemoglobin.

The patient was discharged January 9 and returned periodically for check-up.

	Jan. 19	Feb. 4	Mar. 15	May 9	Oct. 10	Jan. 14	Feb. 3
Erythrocytes	5.75	5.98	5.72	5.08	5.68	6.90	6.12
Hemoglobin	12.3	13.8	12.5	10.6	12.1	13.0	11.0
PCV	39	42	39	34*	37	42	36
MCV	67.8	70.2	68.2	66.9	65.1	60.8	58.8
MCHC	31.5	32.8	32.0	31.2	32.7	30.9	30.5
Sedimentation rate	—	19/+11	17/+6	41/+23	45/+32	29/+21	54/+40
Icterus index	<5	2	5	<5	5	<5	<5
Reticulocytes	0.3	0.3	1.0	0.2	0.1	—	0.1
Nucleated erythrocytes	0	0	1.0	0	0	0	0
Leptocytes	+	+	+	—	—	—	—
Buffy coat	1.5	1.0	1.0 red	1.0 red	2.0	2.0 red	0.5
Leukocytes	19.5	15.2	17.6	21.7	26.6	35.3	3.0
Bands	0	0	0	4.5	9.0	0	0
Neutrophils	56.5	59.0	56.0	58.5	78.5	69.5	83.0
Lymphocytes	12.5	22.0	21.0	18.5	4.0	16.5	7.0
Monocytes	11.0	13.0	8.5	8.5	8.0	10.0	9.0
Eosinophils	20.0	4.0	11.0	10.0	0.5	4.0	1.0
Unclassified cells	0.0	0.5	1.0	0.0	0.0	0.0	0.0
Degenerated cells	0.0	1.5	2.5	0.0	0.0	0.0	0.0

* On standing overnight in the refrigerator a zone of hemolysis appeared above the buffy coat. Fragility in hypotonic saline was tested with beginning hemolysis at 0.51% saline and complete hemolysis at 0.33% saline. Control dog blood was: 0.48% and 0.33% for beginning and complete hemolysis, respectively.

Clinical reports on dates of check-up:

- March 15: Patient has been very active and gaining a little weight. However, on March 9 he showed spasms of a foreleg for the first time.
- May 9: Vomiting. Generalized enlargement of lymph nodes; weeping area of skin in neck region.
- June 2: Owner reports blood in stools periodically. No parasites.
- Oct. 10: Vomiting. Owner thought patient was going into shock. Temp. 102.2° F.
- Jan. 13: Animal in good physical condition; owner states patient is lame in right hind leg. Some blood in urine. Streptococci and micrococci on culture.
- Feb. 12: Patient died. Acute disseminated and suppurative meningoencephalitis; also endocarditis. Several small abscesses (3–4 mm.) in epididymis. Kupffer cells of liver filled with hemosiderin. Bronchopneumonia.

Interpretation:

1. The intense staining of the erythrocytes observed on first admission indicated thickened cells similar to spherocytes. In the wet film the cells appeared dome-shaped and markedly agglutinated. Ghost cells in stained film indicated increased fragility. Occurrence of a zone of free hemoglobin above a buffy coat in the hematocrit was further evidence of increased red cell fragility.
2. The very low level of reticulocytes in the face of extreme anemia would normally suggest depression of erythropoiesis. Bone marrow aspiration revealed a greater than normal red cell production but the punctate basophilia of 13 per cent of the metarubricytes indicated a very unusual and perhaps toxic situation. While erythropoiesis was proceeding at above normal rate, it was ineffective in that viable erythrocytes were not being released to the peripheral blood stream in sufficient quantity to maintain a normal red cell volume.

3. The increased thickness of the red cell suggested a *symptomatic acquired hemolytic anemia* associated with a disease of unknown etiology that started about 5 weeks before. The red cell membrane may have been altered by an autoimmune body which caused the cells to thicken and become susceptible to destruction by the spleen. The blood transfusions may have led to removal of the antibody by adsorption onto the transfused erythrocytes and at the same time the stress on erythropoiesis may have been temporarily relieved by increasing the circulating red cell mass from PCV 7 to PCV 18. The rest to erythropoiesis may have permitted the necessary adjustments within the bone marrow so that more stable erythrocytes were released to the circulation although many were leptocytes (mainly target cells).
4. It is doubted whether the hematinic was necessary. The patient had been on B₁₂, liver injections and high protein diet before coming to the clinic without noticeable benefit. The bone marrow aspiration did not reveal a *megaloblastic* marrow or evidence of iron deficiency. Effective erythropoiesis began about December 10 for on December 14 the first evidence of reticulocytosis and release of nucleated erythrocytes was recorded. The red buffy coat on December 14 was produced by the large number of reticulocytes and nucleated erythrocytes for specific gravity of these immature erythrocytes is about the same as that of leukocytes and thus they may appear in the buffy coat.
5. In the 6 days between December 27 and January 2, the patient was making hemoglobin at a markedly accelerated rate. The hemoglobin increased 4 grams/100 ml. of blood during this period.
6. The erythrocyte sedimentation rate is related to the clumping of red cells between December 3-27. During the period of greatest release of reticulocytes one normally expects the ESR to become less. However, there was marked clumping of red cells during this same period. On May 9 there was a rise in ESR which remained elevated until death 9 months later. This coincided with generalized lymph node enlargement, elevation of WBC and a slight temporary reduction in erythrocytes. This was perhaps the beginning of terminal phase of the patient's case history.
7. The total and differential leukocyte counts were not spectacular during the period of hospitalization. The increase in band neutrophils on December 14 was associated with the reticulocytosis. During the period of repeated check-ups from January 19 to March 15 the leukocyte counts were approaching the maximum normal level and beginning with May 9 the total WBC was observed to increase to considerably above normal. The eosinophils were high on two occasions but this could not be associated with any obvious cause and the monocytes tended to be on the high side throughout.
8. Most spectacular of the leukocyte responses was the marked leukopenia that was detected 9 days before death. Another blood sample was not submitted so it is not known whether this leukopenia persisted. The cause of the leukopenia was not determined but may have been related to a terminal viral infection.
9. Concluding comment to the necropsy report was: Lesions appear to be bacteremic, possibly from valvulitis with secondary manifestations in the central nervous system.

Case 22. Chronic Lead Poisoning in the Canine

A male, wire-haired Terrier, 10½ months of age and weighing about 16 pounds was first seen by the referring veterinarian on October 21. The owner complained that the dog was not eating and displayed signs of nervous irritation. Despite symptomatic treatment and hospitalization on two occasions, the illness continued and vomiting was observed on December 15. The body temperature was within the normal range throughout this period. On December 31, the patient developed running and champing fits (it had been vaccinated for Canine Distemper the previous June). The following day the patient was admitted to the clinic of the School of Veterinary Medicine.

Physical examination revealed an alert, thin, and somewhat dehydrated animal. A routine blood examination revealed a blood picture that was unusual in that there were 53 nucleated erythrocytes per 100 leukocytes but without further evidence of red cell regeneration. In searching for a probable explanation for the high level of nucleated red cells, attention was turned to the spleen. Physical examination had not detected any abnormality of the spleen so a radiograph was requested. The diagnosis became apparent from the radiograph for a dense object was revealed to be present in the stomach. A piece of lead, 1 inch square, was removed surgically. The diagnosis was chronic plumbism.

	<i>Jan. 3</i>	<i>Jan. 5</i>	<i>Jan. 6</i>	<i>Jan. 14</i>	<i>Jan. 26</i>
Erythrocytes	7.45	—	6.75	6.37	6.72
Hemoglobin	14.3	—	13.4	13.5	12.5
PCV	45.0	—	39.0	39.0	39.0
MCV	60.4	—	57.7	61.2	58.0
MCHC	31.7	—	34.3	34.6	32.0
Sedimentation rate	—	—	1/—10	29/+18	1/—10
Icterus index	1.0	—	<5.0	<5.0	<5.0
Reticulocytes	—	1.7	3.2	—	—
Nucleated erythrocytes	53/100 WBC	48/100	89/100	1/100	2/100
Target cells	+++	no record made of erythrocyte morphology Jan. 5, 6, 14			+++
Poikilocytes	++				++
Polychromatophilia	+				+
Leukocytes	15.4/10.0*	25.5/17.3*	34.6/18.0*	28.4	20.3
Metamyelocytes	1.0	—	1.0	0.0	0.0
Bands	3.0	—	10.0	2.0	3.0
Neutrophils	64.0	—	66.0	81.0	74.0
Lymphocytes	18.0	—	16.0	5.0	13.0
Monocytes	10.0	—	3.0	8.0	7.0
Eosinophils	3.0	—	4.0	2.0	3.0
Unclassified cells	1.0	—	0.0	2.0	0.0

* Total leukocyte count must be corrected for nucleated erythrocytes.

Treatment was directed at withdrawal of the lead by administration of the calcium disodium salt of ethylenediaminetetraacetate (CaEDTA). The details of this treatment and results have been reported (J. Am. Vet. M. A., 123: 383, 1953). The patient was discharged on January 30 but it was readmitted to the clinic March 26. Polydipsia, anorexia, and vomiting had persisted during most of this period. On re-entry, the patient was moribund, the pulse rate was 200 and the body temperature was 105° F. The patient died and on necropsy the kidneys revealed tissue changes suggestive of leptospirosis. Chemical analysis indicated high levels of lead in the urine, kidneys, liver and bones.

January 7: Bone Marrow Aspiration.

Myeloblasts	0.2
Progranulocytes	1.5
Myelocytes (neutrophilic)	3.4
Myelocytes (eosinophilic)	0.3
Metamyelocytes (neutrophilic)	3.8
Bands (neutrophilic)	13.8
Bands (eosinophilic)	0.3
Neutrophils	12.8
Total myeloid cells =	36.1
Prorubricytes	1.7
Rubricytes	11.2
Metarubricytes	47.0
Total erythroid cells	59.9
Other cells	4.0
M:E =	0.60:1.0

Note the very high level of nucleated erythrocytes in the blood. This is characteristic of lead poisoning in the dog. Also, note that basophilic stippling of erythrocytes was not observed at any time. The M:E of 0.60:1 indicates active erythropoiesis but the presence of poikilocytes in peripheral blood suggests a shorter life span of erythrocyte. Lead poisoning produces a defect in erythrocyte maturation.

Case 23. Congenital Porphyrria (Pink Tooth) in the Bovine

Calf 1: A Holstein female calf, 3 months of age, was presented with a complaint of skin lesions of 2 months' duration, dark urine, and poor growth. The white areas of the body over the back were scaly, crusty and hairless (photosensitization from porphyrins). The mucous membranes were pale and the teeth were reddish-brown and fluoresced in ultraviolet light. (Uroporphyrin deposition in teeth and bones.) All bones were found to be reddish-brown at necropsy.

Calf 2: A grade, red and white, Holstein female calf, 5½ months old, from another herd was referred with a history of paralysis of 2 weeks' dura-

	<i>Calf 1</i>		<i>Calf 2</i>
	<i>Feb. 4</i>	<i>Feb. 7</i>	<i>March 2</i>
Erythrocytes	2.42	2.55	3.42
Hemoglobin	5.0	4.8	6.7
PCV	15.0	14.0	21.0
MCV	61.9	54.9	61.4
MCHC	33.3	34.2	31.9
Icterus index	< 5.0	—	< 5.0
Reticulocytes	2.8	—	3.6
Nucleated erythrocytes	78/100 WBC	30/100 WBC	32/100 WBC
Erythrocyte morphology in both calves was similar. There was marked polychromatophilia, basophilic stippling, poikilocytosis, and anisocytosis.			
Leukocytes	25.0/14.0 corrected	19.5/14.82	14.0/10.0
Band	0.0	1.0	0.0
Neutrophils	17.0	9.0	25.0
Lymphocytes	58.0	46.0	57.0
Monocytes	24.0	32.0	18.0
Eosinophils	1.0	4.0	0.0
Unclassified cells	0.0	2.0	0.0
Degenerated cells	0.0	6.0	0.0

tion. There were crusty, scaly areas around the nose and rectum; also, alopecia with skin necrosis and scaling over an area of white in the hip region. The teeth and bones were reddish-brown. The urine contained a brownish pigment. The paralysis was due to a fracture of a lumbar vertebra; there were rib fractures also.

Porphyrins: The urines of both calves contained large quantities of the abnormal isomeric uroporphyrin I and a high level of coproporphyrin. These large quantities of porphyrins arise in the bone marrow in the abortive attempt to synthesize hemoglobin.

Interpretation:

1. The RBC, Hb and PCV indicate a well advanced anemia in calf 1 and a moderate anemia in calf 2. The MCV indicates a macrocytic anemia; the mean red cell size is more than double the normal. The large numbers of polychromatophilic erythrocytes, nucleated red cells and basophilic stippling of erythrocytes indicate intense erythropoiesis while the poikilocytosis points to shortened life span of the erythrocytes.
2. Bone marrow from the iliac crest revealed a megaloblastic type of marrow; this agrees with the macrocytic anemia. The nucleated red cells were typically large with basophilic cytoplasm. Some of these large cells contained hemoglobin; it appeared that as soon as hemoglobin deposition started the nucleus was extruded leaving a macrocytic erythrocyte. The marrow was characterized by the large number of free nuclei or hematogones. The Myeloid:Erythroid ratio was estimated to be at 0.1:1. An accurate determination was not possible due to the presence of large numbers of disintegrating blast-like cells and free nuclei.

Case 24. Anemia Associated with Capillary Fragility in the Canine. Possible Vitamin C Deficiency

A German Shepherd male, 3 years old, weighing about 50 pounds, had been bleeding intermittantly from the gums, intestine and urinary tract over a

	Nov. 6	Nov. 9	Nov. 15	Nov. 20	Nov. 29	Dec. 3	Dec. 10
Erythrocytes	3.61	3.12	3.86	4.20	6.43	5.90	5.45
Hemoglobin	6.90	6.30	7.70	9.40	12.20	12.80	11.50
PCV	22.5	20.0	26.0	32.0	42.5	40.5	35.5
MCV	62.3	64.1	67.3	76.2	66.1	68.6	65.1
MCHC	30.6	31.5	29.6	29.4	28.7	31.6	32.4
Sedimentation rate	25/-17	60/+11	48/+14	3/-19	1/-7	6/-3	3/-12
Icterus index	2.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0
Reticulocytes	1.8	1.4	2.1	1.2	0.2	0.1	0.1
Nucleated RBC	0	0	2.0	0	0	0.5	0
Anisocytosis	++	0	+	+	0	+	0
Leptocytes	+	0	0	++++	0	0	0
Polychromatophilia	+++	+	+++	+	0	0	0
Hypochromasia	++	++	+	+	+	0	0
Thrombocytes	nil	0	0	0	nil	decreased	decreased
Buffy coat	0.5	1.0 red	0.5	1.0	<0.5	1.0	0.5
Leukocytes	18.45	25.40	18.65	28.00	8.75	8.20	9.05
Bands	1.5	3.7	2.0	2.0	0.0	0.0	0.0
Neutrophils	78.5	85.0	76.5	85.5	46.5	49.5	67.5
Lymphocytes	7.5	1.7	4.0	3.0	10.0	13.5	9.0
Monocytes	9.0	9.3	13.5	6.5	7.0	11.0	9.5
Eosinophils	2.0	0.3	3.5	3.0	36.5	25.5	13.5
Unclassified cells	1.5	0.0	0.5	0.0	0.0	0.5	0.5

5-month period. The referring veterinarian first saw the patient on October 2 after it had been treated by two other veterinarians without success. At this time the dog exhibited severe depressions, anorexia, oozing hemorrhages at the gums, bloody diarrhea and a temperature of 102.6° F. The PCV was 27 per cent. The patient was treated with whole blood, a total of 1,350 ml. was given in four transfusions between October 2 and October 27. In addition the following were administered: intestinal astringent, streptomycin, albamycin and vitamin K. The patient improved and bleeding stopped up to October 27 when slight blood was oozing from the gums and the patient's well-being deteriorated; 500 ml. of whole blood was given at this time. On October 30 the temperature was 104.6° F., the patient was listless, refused food and it was bleeding from the gums and into the urine. On November 2 the patient was shipped by airplane to the Veterinary School.

Thrombocyte count November 9 = 4,000/cmm. blood; on December 10 = 31,000 per cmm. Due to the apparent low thrombocyte content a clot retraction test also was made on November 9. The test was positive for clot retraction in two hours which appears normal within the limits of the method. B.S.P. liver function test on November 30 revealed normal liver activity.

A urine analysis on November 5 showed many erythrocytes in the sediment and a 4 plus reaction for occult blood.

Clinical record:

Nov. 3—Urine very bloody with clots present. Patient appears normal otherwise. Temperature 101.2° F.

Nov. 4—Blood dripping from prepuce. Temperature 101.4° F.

Nov. P—Bleeding from mouth. Temperature 101.6° F.

Nov. 7—Retinal hemorrhage in left eye. Started treatment with Bioflavinoid and ascorbic acid T.I.D. Gave 200 ml. whole blood, following which, the patient showed signs of shock.

Nov. 8—Stool dark, tarry, some fresh blood.

Nov. 9—Start hematinic (Iberol), continue with Bioflavinoid and ascorbic acid.

Nov. 10—Urine clear. Still fresh blood in mouth. Patient normal otherwise. Start horse meat.

Nov. 12—Mouth bleeding still present. Temperature 102.2° F.

Nov. 13—Some bloody mucous on thermometer. Temperature 101.2° F.

Nov. 21—Very alert, no signs of bleeding.

Nov. 28—Feces still appear dark.

Dec. 6—No more Bioflavinoid and ascorbic acid capsules available. Start vitamin C.

Dec. 10—Returned to owner via United Air Lines. Termination of case unknown. Advised continuing on vitamin C. This case was considered to be a vitamin C deficiency. This is rare since the canine normally synthesizes the necessary vitamin C.

Interpretation:

1. During the period of October 2 to October 27 the referring veterinarian had transfused a total of 1,350 ml. of whole blood. There was only one record of a PCV and that was 27 per cent prior to the first transfusion.

Blood transfusions should be based on actual emergency need. No such need was apparent in this case. In fact the 500 ml. of blood administered on October 27 appeared to have resulted in a delayed transfusion reaction for on October 30 the temperature was 104.6° F. and the patient was depressed. Later on November 7 when another 200 ml. of whole blood was administered the patient is said to have gone into shock. The administration of blood to this patient was over done and could have led to death from a transfusion reaction. It should be recalled that the body makes adjustments for gradual loss of blood so that the volume per cent of erythrocytes or PCV may be reduced as much as two-thirds without placing life in jeopardy.

2. The small buffy coats in the presence of total leukocyte counts of 18,000 to 28,000 are perhaps the result of a very low level of thrombocytes.
3. The negative erythrocyte sedimentation is consistent with the occurrence of a high level of leptocytes.
4. As blood loss was finally controlled between November 15 and November 20, the PCV is seen to increase from 26 to 32 per cent. In the following 9 days it increased another 10 PCV units. After this it began to fall again although the clinical records make no statement about bleeding.
5. The sudden increase in eosinophils on November 29 is not understood.

Case 25. Iron Deficiency Anemia Due to Chronic Blood Loss in the Canine from Heavy Infestation with Stick-tight Fleas

The patient was a 2-year-old, male Springer Spaniel showing extreme weakness, very pale mucous membranes, a body temperature of 99.2° F. and a pulse rate of 168 per minute. The severe anemia was due to blood loss from an extremely heavy infestation with stick-tight fleas. The owner stated that the dog had been sick only 2 days.

	Sept. 18	Sept. 23	Sept. 25	Sept. 28	Oct. 1	Oct. 5	Oct. 12
Erythrocytes	1.94	3.14	3.20	3.86	5.03	6.58	8.09
Hemoglobin	2.25	4.30	5.30	6.30	7.63	8.63	10.80
PCV	9.0	19.0	20.0	25.0	29.0	37.0	38.0
MCV	46.4	60.5	62.5	64.7	57.7	56.2	46.9
MCHC	25.0	22.6	26.5	25.2	26.3	23.3	28.4
Sedimentation rate	diphasic	diphasic	diphasic	23/—13	8/—20	10/—3	0/—12
Reticulocytes	2.3	6.2	6.7	2.3	2.3	—	—
Nucleated RBC/100							
WBC	4.0	2.0	5.0	1.0	1.0	0	0
Anisocytosis	+++	+++	+++	++	++	++	0
Polychromatophilia	++	++	++	0	0	0	0
Hypochromasia	++	+++	+++	+++	+++	+++	+
Leukocytes	25.65	16.40	12.05	7.85	9.10	12.55	11.35
Bands	6.0	5.0	2.0	4.0	7.0	2.0	1.0
Neutrophils	80.0	60.0	56.0	55.0	57.0	60.0	43.0
Lymphocytes	7.0	26.0	38.0	34.0	31.0	26.0	43.0
Monocytes	7.0	6.0	3.0	6.0	3.0	5.0	7.0
Eosinophils	0.0	3.0	1.0	1.0	2.0	7.0	3.0
Unclassified cells	0.0	0.0	0.0	0.0	0.0	0.0	3.0

A transfusion of 250 ml. of blood was administered immediately, the fleas were removed and a hematinic of high iron content was given by mouth. The patient was kept under observation to observe the response of the bone marrow.

Bone Marrow Aspirations from iliac crest

	<i>Sept. 18</i>	<i>Oct. 1</i>
Rubriblasts	0.9	1.3
Prorubricytes	2.5	2.7
Rubricytes	11.8	19.0
Metarubricytes	36.1	43.0
Total erythroid cells	51.3	66.0
Myeloblasts	0.3	0.4
Promyelocytes	0.6	0.5
Myelocytes (neutrophilic)	0.7	1.1
Metamyelocytes (neutrophilic)	2.4	2.4
Bands (neutrophilic)	8.0	9.3
Neutrophils	18.4	8.8
Metamyelocytes (eosinophilic)	0.0	0.3
Bands (eosinophilic)	0.0	0.3
Eosinophils	0.0	0.1
Total Myeloid cells	30.4	23.2
Lymphocyte	4.0	4.1
Monocyte	0.7	0.2
Unclassified cells	2.0	0.5
Degenerated cells	11.6	6.0
M:E =	0.59:1.0	0.35:1.0

Interpretation:

A MCV below 60 cubic microns and a MCHC below 30 per cent indicate a microcytic hypochromic anemia due to iron deficiency. The diet of the animal was not recorded but there is a possibility of low iron intake associated with chronic blood loss. The fact that the owner did not notice that the dog was ill until 2 days before bringing it to the clinic is an indication itself of poor care and attention. The bone marrow aspirations show a predominance of metarubricytes which is typical of iron deficiency. The M:E ratio shows a hyperplastic marrow. The fall of MCV to 46 in the data of October 12 is perhaps related to an error in the erythrocyte count. A count of 7 million would have been more consistent with the rest of the data. This is an excellent example of the speed with which red cell loss can be replaced by a normal bone marrow.

Case 26. Anemia Associated with Hypersplenism in the Canine

The patient was a female Pekinese, about 1 year old, that had been bred a month previously. For about 10 days, it exhibited a partial anorexia, diarrhea and vomiting when it did eat. The physical examination revealed a depressed animal, a large swelling in the abdominal cavity and a body temperature of 100.6° F.

The blood picture on April 4 indicated an anemia in which new erythrocytes were being released as evidenced by erythrocytes with H-J bodies and occasional polychromatophilia and verified by reticulocytes of 2.9 per cent but the red cells were of shortened life span as indicated by the moderate number of poikilocytes. An exploratory laparotomy revealed a markedly enlarged and engorged spleen as the only abnormality. The uterus was gravid. On the assumption that the spleen was responsible for the condition of the patient it was removed. Weight of spleen 51 grams; length 15 cm. An impression smear of the cut surface of the spleen revealed that it was actively

involved in both erythropoiesis and granulopoiesis. Blood examination on April 11 indicated active erythropoiesis and leukopoiesis. A sustained high leukocyte count and the occurrence of target cells are to be anticipated following splenectomy.

	April 4	April 11 (5 days after surgery)	April 18
Erythrocytes	3.70	4.2	5.5
Hemoglobin	8.8	11.3	12.5
PCV	24.0	34.0	38.0
MCV	64.8	80.9	69.1
MCHC	36.6	33.2	32.8
Sedimentation rate	40/+2	not done	not done
Icterus index	< 5.0	< 5.0	< 5.0
Reticulocytes	2.9	1.9	not done
Nucleated RBC/100 WBC	2.0	1.0	1.0
	Occasional polychromatophilia, moderate number H-J bodies, target cells prominent and a moderate number of poikilocytes	Polychromasia moderate, target cells prominent, occasional H-J body.	Target cells prominent.
Buffy coat	3.0*	not recorded	not recorded
Leukocytes	12.90	23.05	26.6
Bands	2.0	0.0	0.0
Neutrophils	75.0	74.0	78.0
Lymphocytes	12.0	15.0	16.0
Monocytes	8.0	8.0	5.0
Eosinophils	0.0	3.0	0.0
Degenerated cells	3.0	0.0	0.0
Unclassified cells	0.0	0.0	1.0

* The lower $\frac{1}{2}$ of the buffy coat was red. A stained film of the buffy coat revealed that the upper white portion was nearly all thrombocytes and lower red portion was an admixture of polychromatophilic erythrocytes and leukocytes.

Histopathological report of splenic tissue: Many nucleated cells, megakaryocytes and large blast-type cells, follicles active.

Subsequent history: The patient was listless on the first day following surgery but improved on the second day and was discharged on the 11th day. A later report stated that the patient made a recovery and whelped normally.

Case 27. Necrobacillosis and Hemorrhage from Abomasal ulcers in the Bovine

A Jersey cow, about 12 years old, was in the dry lot and refused to come to eat on the morning of January 15. A physical examination revealed the mucous membranes to be slightly pale, the feces to be tarry, a body temperature of 100° F., and a pulse rate of 160 per minute. Rumination was absent. The following morning the pulse rate had increased to 180 per minute, the temperature was 98° F., and the feces were black which suggested a gastrointestinal hemorrhage. One gallon of whole blood was administered and the pulse rate fell to 80 in 15 minutes. At 6 o'clock in the evening of the same day, the patient was down and showed extreme depression. The pulse rate was 160 per minute and the rectal temperature was 99° F. The patient was moribund at 10 p.m. and died during the night.

Necropsy revealed ulcers in the abomasum which were the source of the hemorrhage into the gastrointestinal tract. The liver presented miliary abscesses and in addition it was described as being of nutmeg appearance. There

were multiple, foul-smelling abscesses and tracts in the spleen. The lungs were edematous and the trachea and bronchi were filled with frothy fluid. Lesions in the liver appeared to be necrotic foci of *Spherophorus necrophorus* rather than strict abscesses.

	January 15	January 16
Erythrocytes	5.01	4.95
Hemoglobin	8.9	8.4
PCV	25.0	24.0
MCV	49.9	48.5
MCHC	35.6	35.0
Icterus index	15.0	12.0
Reticulocytes	0	—
Nucleated erythrocytes	0	0
Marked rouleaux. Occasional cell shows basophilic stippling.		moderate rouleaux
Buffy coat	3.0 (2/3 red-grey)	3.0 (grey-red)
Leukocytes	43,750	35,900
Progranulocytes	0.3	0.5
Myelocytes	2.0	2.5
Metamyelocytes	4.0	14.0
Bands	16.0	17.5
Neutrophils	37.0	34.0
Lymphocytes	33.0	24.0
Monocytes	3.7	7.0
Eosinophils	2.0	0.5
Unclassified cells	1.0	0.0
Degenerated cells	1.0	0.0
Toxic granules	present	present

Interpretation:

1. The low body temperature in the face of such an extremely high total leukocyte count and very marked left shift with evidence of toxic neutrophils would justify a grave prognosis. This leukemoid blood picture was stimulated by the progressive bacterial invasion of the liver and spleen. Rouleau formation in bovine erythrocytes indicates a grave process.
2. The fact that the animal was bleeding into the gastrointestinal tract led to a certain amount of confusion in the diagnosis. In acute hemorrhage one anticipates an elevation in leukocyte count and left shift but leukocyte counts of this magnitude and shift are not to be expected in hemorrhage. Also in comparing the change in PCV, RBC, and hemoglobin in a fourteen hour period between the blood counts, it is obvious that bleeding was not so severe as to have required a blood transfusion.

Index

A

ABSCCESS, of kidney in canine, case report, 336
 multiple in equine, case report, 341
Achlorhydria, 214, 254
ACTH, adrenal gland activity, test for, 73
 adrenal gland, effect upon, 270
 adrenalectomized animals, effect upon, 270
 experimental injection, in equine, 270
 in inflammation, 277
 leukocyte total count, decreased, 278
Addison's disease, 218
Adrenal cortex, hyperfunction, (Cushing's disease), 218
Adrenal cortical insufficiency, test for, 73
Adrenal gland, ACTH, effect upon, 270
 cortical hormones, effect on lymphocytes, 278
 lymphocytic tissue, 212
 cortical hyperplasia, secondary, 317
 cortical hypofunction (Addison's disease), 218
Agranulocytosis, infectious in feline, 324-325
Anaplasmosis, 257-258
 experimental, case report, 352-353
 hemograms in, 258, 352
 MCV, in remission, discussed, 220
 reticulocytes, in remission, 220
 not parasitized, 221
 transfusion of blood in, 240, 257
Anaplasma marginale, photograph of, 247
 spleen, action upon, 213
 wet blood film, examination for, 54
Anemias, 239-264
 achlorhydria in, 214, 254
 aplastic, *See* hypoplastic, 244, 259-262
 blood loss, classified, 243
 bone marrow in, 106
 cellular response in, 244-245
 in canine, 349
 diet factors in, 221-222
 in equine, a case report, 347
 lack of reticulocytosis in, 122
 in ovine haemonchosis, 353
 blood morphology in, 244-249
 viscosity in, 240
 volume in swine in, 194
 clinical signs in, 239, 240
 compensatory adjustments in, 240
 definition of, 93, 239
 depression type (erythrocytic hypoplasia), 93, 263-264
 in chronic infections, 263, 336, 337, 341
 in chronic interstitial nephritis, canine, 240, 263, 343
 in hypothyroidism, canine, 264, 345
 in malignancy, 263, 282, 339
 in trichostrongylidosis, 264, 354

Anemias, edema in, due to plasma protein reduction, 240
 equine infectious, 355
 erythrocyte sedimentation in, 180
 erythrocyte life span in, 229, 264
 erythrocyte morphology in, 241
 erythropoietin in, 215, 219
 etiologic classification of, 243-244
 feline infectious, 258
 heart murmur in, 240
 hemoconcentration, effect on erythrocytic values, 236, 336-337
 hemolytic types, 254-259
 acquired, 231, 356
 classified, 243
 clinical signs in, 241
 with hemoglobinuria, 254-257
 without hemoglobinuria, 257-259
 hookworm, due to, in canine, 262
 hypersplenism, in canine, 259, 365
 hypoplastic or aplastic, 244, 259-262
 bibliography, 266
 bracken fern poisoning, 260
 definition of, 94
 ionizing radiation, 212, 260
 trichloroethylene-extracted soy bean oil meal poisoning, 260
 hypothyroidism, a cause of, 218, 264, 316-319, 345
 "idiopathic", in canine, 350, 356
 transfusion of blood, value in, 240
 in anaplasmosis, 257-258, 352-353
 in chronic infections, 263
 in coccidiosis, canine, 262
 in leukemia, 263, 282
 in neoplasia of the hematopoietic tissues, 263
 iron deficiency, 74, 254
 in blood loss from flea infestations, canine, 349, 364
 bone marrow characteristics in, 106, 211
 in suckling pigs, 188-189
 iron-dextran injections, for prevention of, 189
 isoimmunization, in newborn, 257
 macrocytic, 241-242
 in nicotinic acid deficiency, canine, 251
 megaloblastic bone marrow in, 242
 transfusion of blood in, 242
 treatment of, 250
 macrocytic normochromic, in congenital porphyria, bovine, 361-362
 in equine myelomatosis, 305
 macrocytic, transitory type, 242
 after blood loss, acute, 74, 347
 anaplasmosis in remission, 352-353
 megaloblastic, in canine, 350

- Anemias, microcytic hypochromic, 74, 242
 in blood loss, chronic, 364
 in copper deficiency, 253
 in hookworm disease, canine, 262
 in iron deficiency, 254
 in pyridoxine (B_6) deficiency, 252
 in riboflavin (B_2) deficiency, 252
 microcytic normochromic, in nephritis, chronic interstitial, canine, 344
 morphologic classification of, 74, 242
 myelophthisic, definition of, 95
 nephritis, chronic interstitial, canine, 263
 normocytic hypochromic, in chronic infection, equine, 341
 normocytic normochromic, 74, 242
 in copper deficiency, experimental in canine, 253
 in distemper, canine, 322
 in hypothyroidism, canine, 264, 316-319, 345
 in infection, chronic, 263
 abscess of kidney, canine, 336
 cellulitis, in canine, 337
 in pyometra, canine, 316
 in infectious anemia, equine, 355
 in leukemia, 263, 282
 in malignancy, 339-341
 in nephritis, chronic interstitial, canine, 263, 312, 343-344
 in the radiation syndrome, 260-262
 in trichostrongylidosis, sheep and cattle, 264, 354
 nutritional deficiencies, 244, 249-254
 bibliography, 264-266
 mineral deficiencies, 253-254
 cobalt, 251
 copper, 253
 iron, 254
 protein deficiency, 221-222
 vitamin deficiencies, 249-253
 ascorbic acid or vitamin C, 252, 362
 B_{12} , 249-251
 folic acid, 251, 252
 niacin or nicotinic acid, 251
 pyridoxine or B_6 , 223, 252
 riboflavin, 252
 parasitism, 264
 bibliography, 267
 blood loss in, 262
 coccidiosis, canine, 262
 lice in cattle, 262
 "Stick-tight" fleas, canine, 262, 349, 364
 trichostrongylidosis, 264, 354
 pernicious type, 96, 211
 in animals, occurrence questionable, 211, 214, 250
 "intrinsic" factor of Castle in, 214
 phenothiazine, as a cause in the equine, 231
 poikilocytes, interpretation of occurrence in, 230
 secondary, 263-264
 bibliography, 267
 classified, 244
 selective depression of erythropoiesis in, 245-246, 249
 hypobilirubinemia in, 227
- Anemias, spherocytosis, a cause of, 231
 treatment of, "idiopathic" types, 240
 Anisocytosis, definition of, 93
 in disease, 245
 in health, in bovine, 115, 153, 160
 in feline, 112
 in ovine, 121
 photograph of, Plate IX-1, 246-247
 Anoxia, erythropoietin in, 215
 Anticoagulants, 34-37
 bibliography, 86
 EDTA, disodium salt of ethylenediamine-tetraacetic acid, 35-37, 47
 leukocyte stainability, effect upon, 35-36
 sedimentation rate, erythrocyte, effect upon, 35
 Heller and Paul formula, 34
 leukocytes, effect upon, 34
 oxalates, ammonium and potassium, 34
 heparin, 34
 oxalate, lithium, for non-protein nitrogen determination, 34
 sodium citrate, for blood transfusion, 34
 "APF" or animal protein factor, 250
 Aplastic, definition of, 93
 Apoferitin in iron utilization, 214
 Arabian horse, brief history, 177-178
 Ascorbic acid or vitamin C, 252,
 deficiency in canine, a case report, 362-364
 folic acid conversion to folinic acid, 252
 in iron absorption, 254
 synthesis by animal species, 252
 Ashby serologic technic, erythrocyte life span studies, 228
 Atomic blast, Bikini, effect on animals, 212
 Azobilirubin, 226
- B
- BACILLARY hemoglobinuria, 255-256
 Banti's disease, 218
 in canine, a case report, 365
 Basophils, description of, in bovine, 116
 in the canine, 111
 in the equine, 125
 in the feline, 115, 149
 in the ovine, 121
 in the porcine, 126
 function of, 269
 normal values, ranges and means, 130, 131
 Basophilia or basophilic, definition of, 93
 Basophilic stippling or punctate basophilia, definition of, 93
 in blood loss or destruction, ovine and bovine, 245
 in anaplasmosis, photograph of, 247
 in congenital porphyria, bovine, 362
 in haemonchosis, ovine, 353
 in metarubricytes, in symptomatic acquired hemolytic anemia, canine, 356
 Bence-Jones protein, absent in myelomatosis of equine, 307
 Bile pigments, in urine, interpretation of, 213
 origin, historical, 226
 Bilirubin, bovine plasma level, 166
 canine plasma level, on high fat diet, 231

- Bilirubin, equine plasma level, 60, 185
 fasting in equine, effect upon, 60, 186
 glucuronic acid, conjugated with, 213, 227
 hepatic regurgitation of, 227
 in erythrocyte life span measurements, 227-228
 in hemolytic disease, 61, 227
 liver, function in removal of, 212-213
 origin of, 226
 reticuloendothelial system, production of, 213, 215, 227
 urine, presence in, 213
 van den Bergh tests for, 226-227
- Biliverdin, production and occurrence, 226
- Black tongue, in the canine, 251
- Blast cell, description of, 102
- Bleeding time, test for, 80
 in canine infectious hepatitis, 323
 in trichloroethylene-extracted soy bean oil meal poisoning, 260
- Blood, 15, 29, 132-134
 bovine, (*See* cow blood).
 canine, (*See* dog blood).
 carbon dioxide combining power of, in nephritis, chronic interstitial, canine, 312-313, 344
 cells, color atlases, bibliography, 129
 nomenclature, bibliography, 129
 clot retraction, test for, 81
 in trichloroethylene-extracted soy bean oil meal poisoning, 260
 clotting time, effect of ionizing radiation upon, 212
 coagulation, process involved and defects, 79-81
 coagulation time, normal values, 80
 capillary tube method, test for, 80
 Lee and White, method of testing, 80
 collection methods, 37-38
 bibliography, 86
 cross matching, simple technic for, 81 83
 film, 40-54
 bibliography, 86
 cover-slip method, 42-43
 examination of, 49-51
 flies, effect upon, 43
 slide method, 41-42
 staining, 43-49, (*See also* stains).
 wet film method, 51-54
 groups, various animals, 81-82
 canine A group, distribution of, 82
 isoantibodies in the canine, 82
 transfusion of blood, dangers of, in animals, 81-82
 in dehydration, 22-24
 bibliography, 28
 leukocytes, means and ranges, various animals, 130, 131
 normal values, ranges and means, 130
 bibliography, general, 196
 general considerations, 132-134
 plasma compartment, 60
 color of, icterus index measurement, 60
 hypobilirubinemia in chronic disease, 227
 protein concentration in anemia, 240
 volume measurement, 17
- Blood, platelet, estimation of, 80. (*See* thrombocytes).
- prothrombin time, ionizing radiation, effect upon, 212
- specific gravity of, in the cat, 150
 in the cow, 166
 in the dog, 142
 in the horse, 186
 in the pig, 196
 in the sheep, 174
- transfusion of blood, 239-240
 repeated, dangers of, 81, 215
- volume, 15
 bibliography, 27
 effect of growth on, in pigs, 194
 estimation from plasma volume, 18
 hemoconcentration, effect upon, 22
 in anemia, 240
 in polycythemia vera, 236
 normal values, various animals, tabulated, 16-17
 discussion of, 21
- Bone marrow, M:E ratio, meaning of, 79
- animals, various, 106-107
 in the cat, 149
 in the cow, 165
 embryonic and post-natal compared, 207
 in the dog, 106, 141
 photographs of cells, 97, 103
 in the goat, 176
 in the horse, 181
 in the pig, 195
 in the pigeon, 209
 in the rabbit, 209
 in the sheep, 173-174
- aspiration, technic, 77-79
 bibliography, 88
 blast cells, occurrence of, 102
 cell identification, versus histologic sections, 210
 in equine, 181, 185
- basophilic stippling, metarubricytes, abnormal in canine, 356
- cellular composition of, 77-78, 97, 103
 bibliography, 129
 degenerated cells, common type in, 102
- erythrocytic maturation series, 101, 103, 210
- granulocytic maturation series, 97, 99, 210
 size of cells, means in sheep, 174
- disease, alteration in, 210
 acquired hemolytic anemia, canine, 356
 acute blood loss or destruction, 244
 in the canine, 106, 365
 cellulitis, in the canine, 338
 congenital porphyria, bovine, 96, 362
 feline panleukopenia, 325
 iron deficiency, 106, 211, 254
 lead poisoning, chronic, in canine, 361
 leukemia, indications for examination of, 298
 canine, granulocytic, 298

- Bone marrow, disease, alteration in, leukemia,
 canine, lymphocytic, 106
 monocytic, 299
 feline, basophilic, 303
 erythroleukemia, 287, 301
 myelomatosis, canine, 300
 equine, 305
 myelophthisic anemia, definition of,
 95
 nephritis, chronic interstitial, canine,
 106
 retained placenta, bovine, 333
 vitamin B₁₂ deficiency, 210
 DL-batyl alcohol content, 208
 bracken fern poisoning, treatment
 with, 260
 dysplasia of, erythrocyte characteristics
 in, 245-249
 erythrocytic hypoplasia of, 263
 erythropoiesis, theories regarding site of,
 208
 evaluation of, 97-107
 fat cells, function of, 77
 granulopoiesis, 274-276
 isotope P³², for study of, 275
 leukocytosis-promoting factor, LPF,
 277
 maturation time, 275
 response in disease, evaluation of, 271
 hematopoiesis, 205
 pre-natal, 207
 hyperplastic, in iron deficiency, 365
 hypoplasia of, in specific diseases, 260
 experimentally produced in the pigeon,
 208
 ionizing radiation, splenic pulp injections,
 value of, 212
 leukocyte reserves in, 275-276
 lymphocytes in, 278
 occurrence of, 102
 source of, 210
 megakaryocytes in, 210
 megaloblastic, 210, 242, 250
 in canine, 211, 350
 in congenital porphyria, bovine, 362
 monocytes in, 102, 210
 neutrophil pool, studies in canine, 275-
 276
 normoblastic, 210
 pernicious anemia type, definition of, 96
 red, distribution of, 77-78
 versus yellow type, 208
 tally sheet, 105
 transplants, 211
 volume, 211
 yellow, composition of, 208
 distribution of, 77
 erythropoietin, effect upon, 219
 Bracken fern poisoning, 260
 Buffy coat, abnormal erythrocytes in, 60
 hematocrit, interpretation of, 59
 red-tinged, interpretation of, 60
 reticulocytosis, effect upon, 60
- C
- CAMALLIDAE family, erythrocyte shape of, 96
 Canine blood, (*See* dog blood).
 Canine distemper, 320-322
 Caprine blood, (*See* goat blood).
 Carbon¹⁴, in erythrocyte life span studies, 228
 in hemoglobin synthesis studies, 224
 Carboxyhemoglobin for hemoglobinometry, 68
 Carotin, carotincids, in bovine blood plasma,
 166
 in equine blood plasma, 186
 Castle, "intrinsic" factor of, 214
 Cat blood, 143-150
 bibliography, 198
 cell counts, capillary versus large vessel,
 effect on, 147
 cells of, described, 112-115
 erythrocytes, changes with age, 147
 diameter of, 148
 fragility of, 148
 Howell-Jolly-like bodies, occurrence of,
 148
 life-span of, 229
 number of, 147
 sedimentation rate of, 148, 238
 sex differences, 148
 hemoglobin values, 147
 icterus index, 150
 leukemia in, 301-304
 leukocyte, total and differential counts,
 149
 total counts, magnitude in disease, 271
 leukocytosis, physiological, 143, 149
 myeloid-erythroid ratio, 149
 neutrophils, Dohle bodies, occurrence of,
 149
 toxic changes in disease, 271
 normal values listed, 130, 131, 143
 from literature, 144-145
 general consideration of, 143
 original data, 146
 reticulocytes, occurrence of, 148
 thrombocytes in, 150
 Cerebrospinal fluid, in monocytic leukemia,
 canine, 300
 Choleglobin, 230
 Cholesterol, lytic inhibitor, 231
 serum level, in hypothyroidism, canine, 317,
 346
 Chromium⁵¹, for erythrocyte life span studies,
 228
 Chylomicron, 54
 Cleaning of glassware, 32-33
 solution for glassware, 32
Clostridium hemolyticum, in hemoglobinuria of
 sheep and cattle, 255
 Clotting time, ionizing radiation, effect upon,
 212
 Cobalt, deficiency of in cattle and sheep, 251
 erythrocyte production, in ruminants, 222
 erythropoiesis, influence upon, 219
 polycythemia from excess of, 222
 Coccidiosis in the canine, 262
 Colostrum, importance of, in isoimmunization
 of the newborn, 257
 Compound F, in inflammation, 277
 Congenital porphyria, case report of, 361
 in cattle, 226
 poikilocytosis in, 230

- Copper, deficiency in ruminants, historical, 253
 molybdenum, relationship to, 253
 erythrocyte production, importance in, 222
 hemoglobin synthesis, relation to, 253
 poisoning, chronic, hemolytic crisis in, 254
- Coproporphyrin, 225
 in congenital porphyria, bovine, 362
 in infection, excretion of, 263
- Coproporphyrinuria, in chronic infection, 263
- Corticosteroids, anti-inflammatory action of, 277
 lymphocytes, action on, 278
- Cortisone, 73
 in inflammation, 277
- Cow blood, 150-166
 bibliography of, 198-201
 bilirubin concentration in, 166
 cells in, described, 115-117
 erythrocytes, basophilic stippling, in calves, 153
 diameter of, 160
 fragility of, 161
 life span of, 229
 number, environmental influences on, 158
 in young bovines, 152
 values in adult cattle, 156-162
 from the literature, 157
 nutrition, influence on, 159
 parturition, effect upon, 160
 sedimentation rate, 161
 seromucoid level versus, 161
 sex differences, 156
 hemoglobin concentration, breed differences, 158
 environmental temperature, effect upon, 159
 icterus index, 166
 leukemia of, 285-293
 leukocytes, adult cattle, 162-165
 counts, total and differential, 162
 differential distribution, age, influence of, 162-163, 292
 drying-off of lactation, effect upon, 164
 estrus, effect upon, 164
 in disease, magnitude of, 271
 pregnancy, effect upon, 164
 in young bovines, 153
 myeloid-erythroid ratio, 165
 neutrophil, band form, significance of, 162
 in disease, percentage indicative of, 162
 toxic changes, 271
 neutrophilia, interpretation of, 271
 normal values, age, effect on, 151-156, 163
 listed, 130, 131, 150, 154, 155, 163
 packed cell volume and hemoglobin, young bovines, 153
 reticulocytes in, 153, 160
 rouleaux formation in, 115, 117, 161, 237, 342, 367
 specific gravity of, 166
 thrombocytes in, 165
- Crenation, reticulocytes, resistant to, 221
- Cushing's disease, 218
- Cushing's syndrome, in the canine, 317
 differentiation from hypothyroidism, 319
- Cyanmethemoglobin for hemoglobinometry, 68
- ### D
- DARE hemoglobinometer, 66
- Dehydration, circulatory system, adjustments in, 23
 clinical signs in, 24
 composition of blood, changes in, 23
 weight loss in, 24
- Desoxyribose nucleic acid, (DNA), leukocytes, life span studies, 275
 mitosis, function in, 219-220
 nucleoli, composed of, 219
- Diffraction for measuring erythrocyte diameter, 74
- Dipetalonema species of microfilaria, 83
- Dirofilaria immitis, microfilaria, 83
- Distemper, canine, 320-322
 bibliography, 333-334
 hemograms in, 321
 inclusion bodies in neutrophil leukocytes, 321
- DL-batyl alcohol, for treatment of bracken fern poisoning, 260
 in yellow bone marrow, 208
- Dog blood, 134-143
 bibliography, 197
 cells of, 107-112
 erythrocytes, age of animal, in relation to, 135
 diameter of, 135
 fragility of, 140
 gestation, effect on number of, 135
 life span studies, 228-229
 sedimentation rate of, 237
 pregnancy, effect on, 140
 table of anticipated values, 139
 sex differences, 135
 hemoglobin concentration, 135
 icterus index, 142
 leukemia in, 293-300
 leukocytes, total and differential counts, 141
 gestation, effect upon, 135
 magnitude in disease, 271
 myeloid-erythroid ratio, 141
 neutrophil, band form, occurrence of, 141
 toxic changes in disease, 271
 neutrophil: lymphocyte ratio, influence of age upon, 141
 normal values, listed, 130, 131, 134
 from the literature, 136, 137, 140
 original data, 138
 reticulocytes in, 135
 specific gravity of, 142
 thrombocytes in, 142
- Döhle bodies, occurrence in neutrophils of cat, 149
- Donkey blood, 186-188
- ### E
- Echinophaga gallinacea*, blood sucking flea, canine, 349

- EDTA for anticoagulant, 35-37
 calcium salt, in treatment of lead poisoning, 360
- Eosinopenia, ACTH, experimental, in horse, 270
 in canine distemper, 322
 in canine Cushing's syndrome, 317
 interpretation of, 274
- Eosinophil, allergic response to drying-off of lactation, bovine, 164
 antigen-antibody reaction, 269
 anti-histamin activity, 269
 at parturition, bovine, 330
 effect on number, 164
 counting of, 73
 description of, in bovine, 116
 in canine, 111
 in equine, 125
 in feline, 115
 in ovine, 121
 in porcine, 126
 function of, 269
 granules, shape, in various animals, 108
 in canine distemper, 322
 in convalescence, 269
 in decomposition of body protein, 269
 in embryonic bone marrow, 207
 in Hodgkin's disease, 282
 in nephritis, chronic interstitial, canine, 345
 normal values, 130, 131
 effect of age on, in bovine, 162
 in canine, 141
 in ovine, 167
 stress, action on, 269
- Eosinophilia, antigen-antibody reactions, 274
 interpretation of, 274
- Eperythrozoonosis, phagocytosis of erythrocytes in, 230
- Equine blood, (*See* horse blood).
- Equine infectious anemia, case report, 355
 erythrocyte sedimentation in, 180
- Erythrocyte, abnormal forms in "buffy" coat, 60
 agglutinated, by Hayem's solution, 70
 in vivo, in trauma, 180
 in wet film method, observation of, 52, 53
 aging, theories of, 230
 anisocytosis in health, in bovine, 115, 153, 160
 in feline, 112
 in ovine, 121
 basophilic stippling of, in bovine and ovine, 245, 247
 in calves, 153
 in congenital porphyria, bovine, 362
 in haemonchosis, ovine, 353
 "bowl-shaped" or "punched-out" forms, occurrence of, in bovine 115
 in ovine, 121
 in porcine, 125
 castration, effect on number, 218
 clumping, in relation to sedimentation rate, 52, 53
 counting, bibliography, 87
 calculations, 71
 cells, pattern for selection of, 71-72
 chamber for, 70
- Erythrocyte, counting, diluting fluids for, 69-70
 Gower's, advantages of, 70
 Hayem's, 69
 error in, 71-73
 in presence of low numbers, 349
 pipettes for, 68-69
 skill in, affect on results, 151, 156
 crenation, cause of, 41
 characteristics of, in canine, 108
 in feline, 108, 112
 in porcine, 108, 125
 reticulocytes resistant to, 221
 description of, in bovine, 115
 in canine, 107
 in equine, 122
 in feline, 112
 in ovine, 121
 in porcine, 125
 destruction of, 229-231
 fragmentation theory, 224, 229
 hemolysis of, 231
 natural lysins for, 231
 replacement rate, canine, 218
 embryonic, 206
 essential materials for, bibliography, 232
 protein, 221
 excitement and exercise, influence on number in horse, 179, 213
 fetal, replacement of, 207, 208
 fragility of, in bovine, 161
 in canine, 140
 in caprine, 176
 in equine, 181
 in feline, 148
 in ovine, 173
 in porcine, 194
 size of cell, influence upon, 85, 175, 223
 test for, 84-85
 fragmentation of, 224
 function of, 223
 ghost cells, 358
 hemolysis, cell size, relation to, 85
 in leukemia, 264
 lytic accelerators and inhibitors, 231
 mechanism of, 223
 species variation, in susceptibility to, 85
 Howell-Jolly body, definition of, 94
 photograph of, 247
 hyperchromasia, indicating spherocytosis, 358
 hypochromic, definition of, 94
 hypoplasia, definition of, 94
 hypotonic moisture, effect upon, 38
 immature, maturation time, hemolytic crisis or hemorrhage, 245
 in Arabian horse, size and number of, 132
 in auto-immunization, 231, 359
 in camallidae (alpaca, llama, camel), shape of, 96, 223
 in cat, changes with age of animal, 147
 in radiation syndrome, 261
 life span, various species, 227-229
 bibliography, 234
 hibernation, effect of, 228
 in deficiencies, nutritional, 229
 in domestic birds, 228

- Erythrocyte, life span, in pigs, 194
 leptocyte, definition of, 94
 (See also, leptocyte)
 macrocyte, definition of, 95
 macrocytic, in vitamin B₁₂ deficiency, 210
 macrocytosis, definition of, 95
 maturation series, 100-101, 103, 210
 MCHC, various animals, 130
 mean size, methods for measurement of, 74-76
 in Mexican deer, 96
 microcyte, definition of, 95
 morphology of, in various anemias, 241
 as determined in wet blood film, 52, 53
 normochromic, definition of, 95
 normocyte, definition of, 95
 nucleated, in feline erythroleukemia, 301
 in health, canine, 107
 in hemolytic crisis, 348
 in pig blood, suckling, 125, 191
 in poisoning, chronic lead, 245, 360
 splenectomy, following, 214
 MCV, various animals, 130
 number, determined from PCV, canine, 59
 in stress, increased, 143
 parasitism, effect on, horse, 179
 in ovine, 167
 parturition and gestation, effect upon in sow, 194
 ovalocyte, definition of, 96
 phagocytosis of, by reticuloendothelial cells, 215
 in haemolymph nodes of bovine, 207
 poikilocyte, definition of, 96
 origin of, 229-230
 polychromatic, polychromasia or polychromatophilia, definition of, 96
 hemoglobin synthesis in, 220, 225
 in equine, not seen in blood loss, 122
 in health, canine, 107
 in pigs, suckling, 125
 Price-Jones curve, in sheep, 173
 protoporphyrin, in chronic infection, 263
 "punched-out" or "bowl-shaped" forms, 246, 247
 reticulocyte, definition of, 96. (See also, reticulocyte).
 rouleau formation, (See rouleaux).
 sedimentation rate of, 55-57, 237-239
 diphasic, definition of, 93
 in piglet blood, 191
 in bovine, 161
 in canine, 139
 in caprine, 176
 in equine, 180
 in feline, 148
 in ovine, 173
 in porcine, 195
 pregnancy, influence upon, 140
 SR/10 formula for equine blood, 181
 table of anticipated values, for correction, canine, 139
 sex differences, in bovine, 156
 in canine, 135
 in equine, 179
 in feline, 148
 Erythrocyte, sex differences, in ovine, 172
 shape of, various animals, 223
 sickled in deer, 223
 site of origin, theories of, 208-209
 size, at birth, bibliography, 88
 in bovine, 152
 in canine, 135
 in equine, 178
 in feline, 147
 in ovine, 167
 in porcine, 191
 versus efficiency, 132, 178
 water intake, influence upon, 160
 species characteristics, 108
 variations, 38, 133
 spherocyte, definition of, 99
 spherocytosis, mechanism of, 231
 spleen, destruction in, 214, 231
 production by, 213, 365
 structure, 223
 bibliography, 233
 target cell, definition of, 94
 in health, in canine, 107
 volume, determination by isotopes, bibliography, 27
 hemoconcentration, effect upon, 22
 measurement of, 19-20
 Wintrobe indexes, 76-77
 Erythrocytometer, Haden-Hausser, 74-75
 Erythrocytosis, definition of, 93
 Erythrogenesis, depression of, in disease, 242
 sedimentation rate, effect upon, 349
 target cells, in response to blood loss, 245
 Erythron, defined, 218
 hormones, influence upon, 218
 Erythropoiesis, 208
 anoxia, influence upon, 218
 bibliography, 232
 cobalt, influence upon, 219
 desoxyribose nucleic acid, function in, 219-220
 embryonic, 205-207
 erythropoietin, importance in, 215
 fundamental stimulus for, 218-220
 in malignancy, 263
 in pigeon bone marrow, 208-209
 in radiation syndrome, 261
 in uremia, experimental, in rabbits, 263
 ineffective, in disease, 358
 minerals essential for, 222
 protein intake, importance of, 221-222
 reticulocyte release, time for formation of, 220
 ribonucleic acid, function in, 219-220
 spleen, action in hypersplenism, 366
 inhibitory action of, 214
 transfusions of blood, effect on, "idiopathic" anemias, 359
 vitamins essential for, 222
 Erythropoietin, 215, 219
 Etioporphyrins, 224
 Exudin, product of inflammation, 277

- Feline infectious anemia, 258. (*See also*,
Haemobartonellosis).
Feline panleukopenia, 324-326
Ferritin in iron metabolism, 214-215
Fetal membranes, retention in bovine, hemo-
gram in, 330-333
Fibrinogen, deficiency of, in canine infectious
hepatitis, 323
in leukemia, granulocytic, in canine, 299
liver, production by, 212
sedimentation of erythrocytes, effect on,
237
Fleas, blood-sucking, in canine, 349, 364
Folic acid, (*See* vitamins).

G

- GIEMSA stain, 43-44, 48
Goat blood, 174-177
bibliography, 201
erythrocyte, diameter, 174
fragility of, 176
general considerations, 132
hemoglobin, 174
leukemia complex of, 307
leukocytes, total and differential counts,
176
myeloid-erythroid ratio, 177
normal values listed, 130, 131, 176
from literature, 175
reticulocytes in, 176
sedimentation of erythrocytes, 176
Gower's solution, for erythrocyte counts, 69
Globulins, abnormal beta, in multiple mye-
loma, equine, 306, 307
alpha, beta, gamma, levels in trichostrongy-
lidoses in the bovine, 354
gamma, lymphocytic origin, 278
Granulocytes, in radiation syndrome, 261
maturation series, 97, 99, 210
spleen, production in, 213
vitamin B₁₂ deficiency, reduction in, 210
Granulocytopenia, DL-batyl alcohol, in treat-
ment of, 208, 260
in bracken fern poisoning, 260
in radiation syndrome, 261
Granulopoiesis, 208, 274-276
in disease, effectiveness of, 271
leukocytosis-promoting factor, in inflam-
mation, 277
spleen, in hypersplenism, 366

H

- HADEN-HAUSSER erythrocytometer, 74-75
Haemobartonella felis, 247, 258
wet blood film examination for, 54
Haemobartonellosis in feline, 258
leukemia, confusion with, 301
phagocytosis of erythrocytes in, 230
spleen, action on, 231
removal, effect of, 213
transfusion of blood in, 240
Haemolymph nodes, bovine, 207
Haemonchosis in cattle and sheep, 262, 353
poikilocytes, occurrence in, 230
Hayem's solution, for erythrocyte counts, 69
Hematinic, in "idiopathic" anemia, value of,
240, 242, 350

- Hematinic, secondary anemias, not indicated,
242
Hematocrit, 93, (*See also*, Microhematocrit).
anticoagulants suitable for, 34-35, 54
"body", ratio to "venous", 20
bibliography, 26
buffy coat, 59-60
in disease, chronic, 246
in thrombocytopenia, low, 364
reticulocytes in, red appearance, 359
thrombocytes, in bovine, 116
centrifugation, duration of time required,
58, 156
force required, 57-58
cleaning of, 33
comparative value of, 29
icterus index in, 60
Koepe's criterion for complete packing, 59
packed cell volume, PCV, 59
ranges and means, various animals,
130
plasma compartment, 60
hemolysis above buffy coat, signifi-
cance of, 358
"trapped" plasma, 18, 59, 76
factors influencing trapping, 18-19
Wintrobe method, 54-61
bibliography, 86-87
"venous", 18
bibliography, 26
correction factor for, 20
Hematogone, definition of, 105
in congenital porphyria, bovine, 362
Hematopoiesis or hemopoiesis, 205-217
bibliography, 216-217
embryonic and early post-natal, 205-207
in blood loss, 349
in haemolymph nodes of bovine, 207
in liver, 212
lymph nodes and follicles, 212
radiation syndrome, effect upon, 261
Hematoxylin, for testing pH of water in stain-
ing, 44
Hematuria, in canine, 348
Heme, 224, 225
Hemochromatosis, 215
Hemoconcentration in disease, 22-24, 236, 322
in case reports, 335, 337
sedimentation of erythrocytes, effect upon,
238
Hemocytometer, 68-73
cleaning, 33
inherent errors, 71
Hemoglobin, bilirubin from, 226
catabolism in R:E system, 214, 215
composition, 64
concentration, ranges and means, various
animals, 130
altitude, effect of, in sheep, 172
nutrition and parasitism, effect on, in
sheep, 167
correlation with specific gravity of blood,
166
destruction, 226-227
fragmentation of erythrocytes, effect on,
224, 229
from bile pigments, historical, 226
in suckling period of growth in, calf, 153
kitten, 148

- Hemoglobin, in suckling period of growth in,
piglets, 188-190
iron, content, 64
from erythrocyte destruction, 215
MCH, ranges and means, various animals,
130
MCHC, ranges and means, various animals,
130
molecular weight, 64
osmotic pressure of, 224
oxygen capacity, 64
packed cell volume, derived from, 59
synthesis, 224-226
bibliography, 233
diet factors, essential for, in canine, 222
copper, 253
protein, 221
pyridoxine, 252
in blood loss, chronic, 252
in infection, chronic, 263
in immature erythrocytes, circulating,
220, 225
in iron deficiency, 254
in rubricytes, 105
temperature, environmental, effect upon,
in cattle, 159
Hemoglobinemia, 61, 226
influence upon MCHC value, 348, 357
Hemoglobinometry, 64-68
bibliography, 87
methods for, 65
acid hematin, 66
carboxyhemoglobin, 68
cyanmethemoglobin, 68
automatic pipette for, 67
Dare, 66
oxyhemoglobin, 66
photoelectric, 66
Spencer Hb meter, 66
Tallqvist, 66
Hemoglobinuria, 226, 241
in bacillary hemoglobinuria, bovine, 255
in copper poisoning, chronic, 255
in "idiopathic" hemolytic crisis, canine, 348
in isoimmunization of the newborn, 257
in leptospirosis, 256
in post-parturient period in cattle, 255
in trichloroethylene-extracted soy bean oil
meal poisoning, 260
in water intoxication, calves, 255
Hemograms in disease, appendix, case re-
ports, 335-367
bovine, anaplasmosis, experimental, 258,
352
bacillary hemoglobinuria, 256
fetal membranes retained, 332
lymphosarcoma, 289, 290
mastitis, acute, 329
traumatic reticulitis, 327
canine, distemper, 321
hypothyroidism, 318
infectious hepatitis, 323
leptospirosis, 320
leukemia, lymphocytic, 294-297
monocytic, 300
nephritis, chronic interstitial, 313
pyometra, 314-315
Hemograms, canine, radiation syndrome, 262
feline, leukemias, 302
panleukopenia, 325
equine, myelomatosis, 307
Hemograms, normal, various animals, 130, 131
bovine, normal, relation to age, 154, 155
from the literature, 157, 163
canine, clinically normal, 138
from the literature, 136-137, 140
caprine, from literature, 175
donkey and mule, from literature, 187
equine, all ages, from literature, 182-184
before and after exercise, 213
feline, clinically normal, 146
from the literature, 144-145
porcine, suckling, 190
all ages, 192-193
sheep, immature and parasite-free, 172
from literature, 168-171
Hemolysis, erythrocytes, over-aged, 231
fats and soaps, as a cause, 61, 231
in leukemia, 264
lysis, natural, occurrence of, 231
accelerators of, 231
mechanism of, 223, 224
naphthalene (moth balls), 231
phenothiazine, action in, 231
reticulocyte, resistant to, 221
Hemolytic disease, Appendix cases, 347, 356
auto-immunization, a cause, 231, 356
bibliography, 266
bilirubin, type in, 227
clinical signs in, 241
erythrogenesis, evidence in peripheral blood,
211
icterus index in, 61
leukocytosis in, 244, 349
neutrophilia in, 273
pyrexia in, 242, 244, 353
shift to left in, 244, 349
splenic function in, 231
Hemorrhage, blood restoration following, 15,
24-26
blood volume, effect upon, bibliography, 28
chronic, effect on blood volume, 239
clinical signs, relation to rate of blood loss,
25
erythrogenesis, evidence of, peripheral
blood, 211
experimental in dogs, survival rate, 25
hemodilution following, 25
hemoglobin replacement following, in can-
ine, 222
hemosiderin in, 215
in bracken fern poisoning, 260
in hookworm disease, 262
in trichloroethylene-extracted soy bean oil
meal poisoning, 260
in vitamin C deficiency, canine, 363
into abdominal cavity, failure of clotting
mechanism, 270
neutrophilia in, 274
plasma protein, changes following, 25
plasma volume, rate of replacement, 25
shock, death from, following, 25
occurrence in, 239
vascular system adjustments in, 349

- Hemosiderin, 215
- Hemostatic defects, bibliography, 88
in canine infectious hepatitis, 323
tests for, 79-81
- Heparin, 34-35
in basophils, 269
in canine infectious hepatitis, 323
- Hepatic, (*See* Liver).
- Hepatitis, canine infectious, 323
- Heterophil, 99, 269
- Hibernation, in radiation syndrome studies, 262
- Hodgkin's disease, 282, 300
- Hormones, influence on erythron, 218
- Horse, Arabian, brief history, 177-178
Quarter, brief history, 178
Thoroughbred, brief history, 177
- Horse blood, 177-186
bibliography, 200, 202-203
cells of, 122-125
erythrocytes, age of animal, influence upon, 178-179
agglutinated *in vivo* following trauma, 180
diameter, 179
fragility, 181
life span, 229
number, PCV and hemoglobin, 178
excitement, influence upon, 179, 213
exercise, influence upon, 213
hemoglobin saturation, possible relationship to performance, 178
"hot-blooded" and "cold-blooded" breeds compared, 178
in Arabian, 132, 183
parasitism, effect upon, 179
sedimentation rate, 180-181
sex, pregnancy, lactation, and barrenness, effect upon, 179
general considerations, 177-178
icterus index, 185
leukemia complex in, 304-307
leukocytes, age of animal, influence upon, 181
magnitude of response, in disease, 271
total and differential, 181
Myeloid-erythroid ratio, 181, 185
neutrophil-lymphocyte ratio, 181
normal values listed, 130, 131, 177
from literature, 182-184
reticulocytes, lack of in, 180, 211, 347
rouleaux, 122, 237
specific gravity of, 186
thrombocytes in, 185
- Howell-Jolly bodies, 94, 245
in haemonchosis, ovine, 353
in health, occurrence of, in canine, 107
in equine, 108, 122
in feline, 108, 112, 148
in porcine, 125, 191
photograph of, 247
- Hydrochloric acid, in iron utilization, 214
- Hyperchromasia or hyperchromatic, definitions of, 94
- Hypochromasia, hypochromatic or hypochromic, definitions of, 94
- Hypochromasia, in acquired hemolytic anemia, canine, 358
photograph of, 247
- Hyperchromic, definition of, 94
- Hypersegmenter, definition of, 94
- Hypersplenism, 259, 365
- Hypobilirubinemia in chronic diseases, 227, 344
- Hypoferremia in infection, 263
- Hypoproteinemia, effect on hemoglobin synthesis, 221
in trichostrongylidosis, bovine, 354
- Hypothyroidism in canine, 264, 316-319
bibliography, 333
case report, 345-346
differentiation from Cushing's syndrome, 319
hemograms in, 348
tests for, 317
- I
- ICTERUS, 227
in infectious hepatitis, canine, 322
in leptospirosis, canine and bovine, 256
- Icterus Index test, factors influencing plasma color, 60-61, 166
interpretation of, in various animals, 60
in bovine, 166
in canine, 142
in feline, 150
in equine, 185
in porcine, 196
- "Idiopathic", definition of, 94
- Inclusion bodies, in canine distemper, 320, 321
in infectious canine hepatitis, 323
- Infectious canine hepatitis, 322-324
- Inflammation, bibliography, 279-280
biology of, 276-278
- Intrinsic factor of Castle, 249-250
- Iron, absorption, theory of, 214-215
ascorbic acid, function in absorption of, 254
phosphorus, relationship to absorption of, 254
apoferritin, function in utilization of, 214
ferritin, function in utilization of, 214
hemoglobin content of, 64
in molecule of, 224-226
synthesis failure in hypoproteinemia, 221
- hemosiderin, 215
- hydrochloric acid, importance in absorption of, 214
- hypoferremia in chronic infection, 263
- in erythrocyte, life span studies, 228
in production of, 222
- in milk, sow's, content of, 188
- metabolism of, 214-215
- pig, body content at birth, 188
suckling, needs, 188-189
- pyridoxine or B₆, in utilization of, 252
- reducing agents in gut, influence on absorption of, 214
- soil, concentration in, 189

- Iron deficiencies, anemia in, 74, 254
 - due to blood loss, chronic, in canine, 364
 - hydrochloric acid, relationship to, 254
 - metarubricytes of bone marrow in, 211
 - microcytic-hypochromic anemia, typical of, 242
- Iron pyrophosphate, for iron deficiency medication, piglets, 189
- Iron-dextran, for iron deficiency medication, piglets, 189
- Iron sulfate, for iron deficiency medication, piglets, 189
- Isoimmunization in the newborn, 81, 257

K

- KIDNEY, abscess in canine, 336-337
 - erythropoietin production by, 219
 - cobalt, effect upon, 219
- in hematopoiesis, 205, 215, 219
- in leptospirosis, 320
- necrosis, action upon, 277
- Koeppé's criterion for complete erythrocyte packing in the hematocrit, 59

L

- LEAD poisoning, chronic, in canine, 360
- Leptocyte, 94, 245, 247
 - bibliography, 264
 - "folded-cell" type, in canine, 247, 249
 - in "buffy" coat of hematocrit, 60, 246
 - in hypothyroidism, canine, 319, 346
 - in hypotonic saline, resistance to, 249
 - in splenectomy, 214
 - rouleaux, participation in, 246
 - sedimentation rate, erythrocyte, altered by, 238, 346, 364
 - target cell type, in canine, 247, 249
- Leptospirosis, anemia in, 256
 - bibliography, 333-334
 - hemogram in, 320
 - in canine, 319-320, 343
- Leukemia, 281-311
 - antimetabolites for folic acid, in treatment of, 252
 - bibliography, 309-311
 - definition of, 94
 - Di Guglielmo's syndrome, 282
 - etiology, facts and theories, 283-285
 - genetic influences, 283
 - ionizing radiation, 284
 - in atomic blast survivors, Hiroshima, 284-285
 - in radiation syndrome, latent development of, 261
 - transmissible agent in, 283
 - Hodgkin's disease, differentiation from, 282
 - in man compared to the disease in animals, 281-283
 - myelogenous, (*See* Granulocytic).
- Leukemia complex, bovine, 285-293
 - bibliography, 309
 - Federal reports, incidence of, 290
 - granulocytic type, 285

- Leukemia complex, lymphocytic cell type, lymphosarcoma, 286-293
 - age relationship in, 292
 - in a fetus, 285
 - in calves, 286
 - anemia, occurrence of, 290
 - clinical signs in, 286
 - hemogram in, 289, 290
 - abnormal lymphocytes in, 289
 - leukopenia in, 290
 - herd problem of, 290-293
 - Bendixen's key, for detection of, 291
 - Goetz's key, for detection of, 291
 - mammary gland involvement, 286
 - California mastitis test positive reaction in, 286
- Leukemia complex, canine, 293-300
 - basophilic cell type, 300
 - bibliography, 310
 - granulocytic cell type, 293, 298-299
 - Hodgkin's disease, so-called, 300
 - lymphocytic cell type, 293-298
 - anemia in, 264, 298
 - bone marrow in, 106
 - clinical signs in, 293
 - monocytic cell type, 293, 299-300
 - myelomatosis, 300
- Leukemia complex, equine, 304-307
 - bibliography, 311
 - myelomatosis, 305
- Leukemia complex, feline, 301-304
 - anemia, occurrence of, 264, 301
 - basophilic cell type, 303
 - bibliography, 310
 - erythroleukemia, 301
 - haemobartonellosis, confusion with, 301
 - pyrexia in, 301, 302
- Leukemia complex, lymphosarcoma, caprine, 307
- Leukemia complex, ovine, 308
 - bibliography, 311
- Leukemia complex, porcine, 308
 - bibliography, 311
- Leukemoid, definition, 95, 272
 - in abscess of kidney, canine, 336-337
 - in pyometra, canine, 315-316
- Leukocyte, 268-270
 - azurophil granules in, 91
 - bibliography, 279-280
 - blast cell, description of, 92
 - circulating, ratio to non-circulating, 276
 - classification, principles of, 91-92
 - clumping in peripheral blood, meaning of, 269
 - counting of, method and equipment, 68-73
 - bibliography, 87
 - correction for nucleated erythrocytes, 71
 - error of, 71-73
 - differential count of, 49-51
 - means and ranges, in absolute numbers, 131
 - means and ranges, absolute and relative numbers defined, 272
 - in absolute numbers, 131
 - in percentage, 130

- Leukocyte, differential count of, number to be differentiated, 51
 reliability of, 50
 disintegrated cell, description of, 92
 elimination of, from the body, 268
 eosinophil, direct counting method, 73
 granulocytes, life span, 274-275
 maturation series, 97-100
 pernicious anemia type, defined, 96
 heterophil, 99, 269
 hormones, influence upon, 270
 in bovine, estrus, pregnancy, and parturition, effect on, 164
 in disease, classification of response of, 272
 magnitude of response, in various animals, 271-272
 in inflammation, effect of pH on, 277
 in parturition, in swine, 195
 leukemoid distribution of, 272
 metamyelocyte, description of, 99
 occurrence post-partum, in swine, 126
 monocyte maturation series, description of, 100
 myeloblast, description of, 99
 myelocyte, description of, 99
 neutrophil, description of, 100
 band form, 99
 sex bud, characteristic of female, 107, 109, 113, 115
 toxic, definition of, 99
 neutrophil-lymphocyte ratio, various animals, 108
 number in peripheral blood, age, effect of on, in bovine, 153, 163, 292
 "buffy" coat of hematocrit, relation to number of, 59
 meaning of, 275
 origin and function of, 269-270
 progranulocyte, description of, 99
 shift, left, definition of, 96
 degenerative, 96, 272
 regenerative, 96, 272
 in parturition in swine, 195
 in sheep, 173
 shift, right, definition of, 99
 special features in, various species, 108
 stress, character of, in bovine, explained, 278
 response to, 133, 271
 terminology, standardization of, 91
 toxic manifestations, in canine, 111
 transfusion of, 268
 Leukocyte-promoting factor, LPE, 277
 Leukocytosis, 274
 "buffy" coat, evidence of, 60
 in abscess of kidney, canine, 336
 in blood loss, acute, 347
 in canine distemper, 322
 in cat blood, neutrophil percentage, 149
 in cellulitis in the canine, 337-338
 in feline panleukopenia, 325
 in hemolytic crisis, 244
 in infectious hepatitis, canine, 324
 in leptospirosis, 319
 in necrobacillosis in the bovine, 366
 in parturition, bovine, 164, 330
 in pyometra, canine, 316, 338-339
 Leukocytosis, in sheep, 173
 in traumatic reticulitis, bovine, 326-328
 in wet blood film, examination for, 51, 53
 non-specific, in canine lymphosarcoma, 293
 physiologic, 143, 152
 following exercise, in equine, 213
 Leukopenia, 274
 "buffy" coat, evidence of, 60
 in canine distemper, 322
 in infections, overwhelming, 276
 in infectious hepatitis, canine, 323, 324
 in leukemia complex, 290
 in mastitis, acute bovine, 328-330
 in multiple myeloma, equine, 306
 in nicotinic acid deficiency, canine, 251
 in panleukopenia, feline, 324-326
 in radiation syndrome, 261
 in sulfanilamide therapy, 252
 in viral diseases, 274, 355
 in vitamin B₁₂ deficiency, 211, 250
 in wet film, examination for, 51, 53
 Leukopenin, in inflammation, 277
 Leukopheresis, 275
 Leukotaxine, in inflammation, 276
 Lipemia, 51, 54, 61
 Liver, as leukocyte sequestration site, 268
 bilirubin excretion, 212-213, 227
 cirrhosis, due to excess iron, 215
 copper storage, hemolytic crisis in, 254
 fibrinogen, production of, 212
 hematopoiesis in, 205-206
 hepatitis in cellulitis, canine, 337-338
 inclusion bodies in canine infectious hepatitis, 323
 necrosis, in inflammation, action on, 277
 prothrombin production, 212
 Lymphoblast, description of, 100
 Lymphoblastoma, lymphocytoma, (*See* Leukemia complex).
 Lymphocyte, 100, 270, 278-279
 adrenal gland, action on, 212, 278
 antibody formation by, 270
 at parturition, in the bovine, 330
 binucleate forms, in ovine, 121
 bone marrow, occurrence in, 102, 210
 counts of, absolute, age influence on, in bovine, 292
 in relation to magnitude of leukocytosis in disease, 278
 description of, in bovine, 116
 in canine, 111
 in equine, 125
 in feline, 115
 in ovine, 121
 in porcine, 126
 embryonic formation of, 206-207
 immune response, function in, 278
 in convalescence, 270
 in infection, initial phase, 270
 in leukemia of bovine, described, 289
 in radiation syndrome, 261
 life span studies, 279
 lymphopenia, 274
 absolute, in canine distemper, 322
 in nephritis, chronic interstitial, canine, 212, 312, 344, 345
 ACTH, production of, 270

- Lymphocyte, lymphopenia, in Cushing's syndrome, canine, 317
 in infectious hepatitis, canine, 323
 in mastitis, acute bovine, 328
 in radiation syndrome, 261
 relative, described, 273
 maturation series, description of, 100
 number, normal values of, 130, 131, 278
 origin of, 269
 pH, effect upon in inflammatory exudate, 277
- Lymph nodes and follicles, in hematopoiesis, 212
- Lymphoma, 285
- M
- MACROCYTE, macrocytic, macrocytosis, defined, 94
- Malignant lymphoma, (*See* Leukemia complex).
- Mast cell, 269, 270
- Mastitis, bovine, acute, 328-330
 bibliography, 334
- MCH, or mean corpuscular hemoglobin, formula, 77
- MCHC, or mean corpuscular hemoglobin concentration, formula, 77
 free hemoglobin in plasma, influence upon, 348, 357
 in anemia, morphologic classification of, 242
 in blood loss, evidence of intensified erythrocytogenesis, 245
 in iron deficiency anemia, canine, 365
 microhematocrit, influence on, 77
 ranges and means, various animals, 130
- MCV, or mean corpuscular volume, formula, 76
 accuracy of, 76
 erythrocytogenesis, as evidence of, 245, 348
 in anaplasmosis in remission, 258, 352
 in blood loss, haemonchosis, in ovine, 353
 in iron deficiency anemia, 364-365
 morphologic classification of anemia, use in, 242
 ranges and means, various animals, 130
 reticulocytosis, effect on, 220
- Megakaryoblast, description of, 100
- Megakaryocyte, description of, 100
 in bone marrow, mammals, 210
 in embryonic development, 206-207
 spleen, production in, 213
 thrombocytes from, 209-210
- Megaloblast, anemia, megaloblastic, canine, 350
 definition of, 95
 in congenital porphyria, bovine, 362
 in maturation arrest in bone marrow, 210, 220, 250
 in vitamin B₁₂ and folic acid deficiencies, 219, 250
- Metarubricyte, denucleation of, in embryonic bone marrow, 207
 description of, 101
 in iron deficiency or chronic blood loss, 211, 365
- Metamyelocyte, description of, 99
 in embryonic development, 207
 in equine peripheral blood, occurrence of, 122
 in swine, peripheral blood, post-partum, 126
 in vitamin B₁₂ deficiency, giant forms of, 210-211
- Microcyte, microcytic, microcytosis, defined, 95
 from fragmentation of erythrocytes, 230
- Microfilariæ, bibliography, 89
 differentiation of, in canine blood, 83
 methods of examination of blood for, 83-84
 wet blood film, presence of, 54
- Microhematocrit, 61-64
 accuracy of, 61
 bibliography, 87
 centrifuge for, photograph of, 62
 MCHC, effect upon, 77
 readers for, examples shown, 63
- Microscope, discussion of optics, 30
 obtaining correct illumination, 31
- Monoblast, description of, 100
 bone marrow, presence in, 102, 210
- Monocyte, description of, 100
 differentiation from myelocytes, 112
 in bovine, 116
 in canine, 112
 in chronic infections, 270
 in equine, 125
 in feline, 115
 in lymphosarcoma, equine, 305
 in ovine, 122
 in porcine, 126
 maturation series, description of, 100
 normal values, ranges and means, various animals, 130, 131
 origin of, 269
 R-E system a possible source, 215
 types I and II in canine blood, 112
- Monocytic leukemia, canine, 299
- Monocytosis, 273, 274
 in disease, evidence of chronicity, 274
 in abscess of kidney, canine, case report, 336-337
 in cellulitis, canine, case report, 337-338
 in distemper, canine, 322
 in infectious hepatitis, canine, 323
 in pyometra, canine, 316, 338
 in retained placenta, bovine, 331-332
- Molybdenum, chronic poisoning, bovine, 253-254
- Mule blood, 186-188
- Myelocyte, description of, 99
 homoplastic nature of, 102
 in embryo, 207
- Myelogenous leukemia, (*See* Leukemia complex, granulocytic).
- Myeloid-Erythroid ratio 106-107
 age, changes with, 165
 in bovine, 165
 in calves, 207
 in canine, 141
 in caprine, 176
 in equine, 185
 in feline, 149
 in ovine, 174

- Myeloid-Erythroid ratio, in porcine, 195
 Myelomatosis or myeloma, multiple in canine,
 300
 in equine, 305

N

- NAPHTHALENE, a cause of hemolysis, 231
 Necrobacillosis, in bovine liver, 366
 Necrosis, a product of inflammation, 277
 Neoplasia, anemia, type of, 263
 Nephritis, acute, in leptospirosis of the canine,
 320, 343
 chronic interstitial, canine, 312-313
 anemia, depression type, 240, 263
 bibliography of, 333
 bone marrow in, 106
 case reports of, 343, 344
 hemograms in, 313
 hypobilirubinemia in, 227, 344
 lymphocytic tissue, absolute lympho-
 penia, characteristic of, 212,
 344, 345
 effect upon, 212
 osteodystrophy or "rubber jaw" in,
 313
 poikilocytes, occurrence of, 230
 Neutropenia, in hypersplenism, 259
 in infectious hepatitis, canine, 323
 in mastitis, acute, bovine, 328
 in sheep, 173
 in vitamin B₁₂ deficiency, piglets, 250
 Neutrophil, band form, importance to dis-
 tinguish properly, 99
 occurrence in peripheral blood, inter-
 pretation of, 105
 description of, 100
 heterophil, alternate term in animals, 269
 hypersegmented, definition of, 94
 in vitamin B₁₂ deficiency in man, 211
 in embryonic development, 206-207
 normal values, ranges and means, various
 animals, 130, 131
 phagocytosis by, 269
 pH, in inflammatory exudate, effect upon,
 277
 sex bud, nuclear, indicative of female, 107,
 109, 113, 115, 121
 shift to the left, 96
 degenerative, 272, 335
 regenerative, 272, 244, 336, 337, 341,
 348
 toxic forms, 99, 271, 274
 Neutrophilia, 274
 ACTH, in experimental injection, 270
 in blood loss acute or destruction, 347, 348
 in bovine, at parturition, 164
 in traumatic reticulitis, 326, 328
 interpretation of 271-272
 in canine, distemper of, 322
 in leptospirosis, 319, 343
 in nephritis, chronic interstitial, 312
 in equine, in lymphosarcoma, 304, 305
 in malignancy, 340-341
 with left shift not prominent, 122
 in ovine, 173
 stimuli, various causing, 273

- Neutrophils, bovine, 116, 162
 with left shift, occurrence and significance
 of, 162, 272
 in indigestion, acute, 326
 in lymph nodes, formation of in
 in calves, 206
 in mastitis, acute, 328-330
 in necrobacillosis of liver, 366-367
 in retained placenta, 330-331
 in traumatic reticulitis, 326-328
 toxic granulation in, 116
 Neutrophils, canine, 97, 107, 111, 141
 inclusion bodies in, in canine distemper,
 321
 "pool" of, for immediate response to de-
 mand on, 275
 toxic changes in, in leptospirosis, 343
 in peritonitis, 335
 with left shift, degenerative, in peritonitis,
 335
 regenerative, in abscess of kidney, 336-
 337
 in cellulitis, 337-338
 in pyometra, 316, 338-339
 Neutrophils, equine, 122, 123
 with regenerative left shift, 341-342
 Neutrophils, feline, 112-115
 angular granules or Döhle bodies, oc-
 currence of, 115, 149
 left shift, in panleukopenia, in conva-
 lescence, 325
 leukocytosis, relation to, 149
 Neutrophils, ovine, 121, 167, 171
 Neutrophils, porcine, 125, 195
 with left shift, 195
 Neutrophils, rabbit, called pseudo-eosinophil,
 209
 Neutrophil:Lymphocyte ratio, various ani-
 mals, 108
 Nitrogen¹⁵, experimental use, in heme syn-
 thesis, 224
 in life span studies, erythrocyte, 228
 Non-protein nitrogen, in nephritis, chronic
 interstitial, canine, 312, 344, 345
 in pyometra, canine, 316, 338
 levels in, leptospirosis, canine, 320, 343
 Normocytic, normochromatic, normochro-
 masia, defined, 95
 Nutritional deficiency anemias, 249-254

O

- OSTEODYSTROPHY in nephritis in the canine,
 313
 Ovalocyte, definition of, 96
 Ovine blood, (*See* Sheep blood).
 Oxalate, as anticoagulants, 34
 crystals ingested by neutrophils, 126

P

- PANLEUKOPENIA, bibliography, 333-334
 feline, 324-326
 hemograms in, 325
 Para-amino-benzoic acid, relationship to
 folic acid and antimetabolites, 252

- Parasitism, anemia in, 262
 bibliography, 267
- Parathyroid glands, enlargement in nephritis, canine, 345
- Parturition in bovine, hemograms, 332
- PBI, protein bound iodine, test for hypothyroidism, 317
- PCV, packed cell volume, in hematocrit, 57-60
- Pernicious anemia, 96, 211, 250
- Phagocytosis, of bacteria, by neutrophils, 269
 of erythrocytes, by immature neutrophils, 269
 by monocytes in certain parasitic diseases of the blood, 230
 by the R.E. cells, 215
 in haemolymph nodes of cattle, 207
- Phenothiazine, mechanism of anemia produced, 231
- Phosphate buffer, in staining of blood film, 45
- P³², in life span studies of leukocytes, 275
- Phosphorus deficiency, high diet level, decreases iron absorption, 254
 in post-parturient hemoglobinuria, bovine, 255
- Photosensitization, in congenital porphyria, bovine, 361
- Pig blood, 188-196
 bibliography, 203-204
 erythrocyte, fragility, 194
 life span, 194, 229
 number, 191
 gestation and parturition, effect on, 194
 sedimentation rate, 195, 238
 size, 191
 hemoglobin concentration, various ages, 194
 icterus index, 196
 leukemia complex, 308
 leukocytes, total and differential, 195
 at parturition in swine, 195
 magnitude in disease, 271
 normal values listed, 130, 131, 188, 190, 192-193
 various factors, effect upon, 192-193
- Myeloid:Erythroid ratio, 195
- PCV and blood volume, 194
- reticulocytes, 191
- specific gravity, 196
- thrombocytes, 196
- young or growing animals, 189-191
 age and husbandry, influence on values, 190
 erythrogenesis, evidence of in, 191
 iron requirements, 188-189
 sedimentation of erythrocytes, diaphasic reaction, 191
- Pig stomach, "intrinsic" factor of Castle, 214
- Pipettes, blood cell counting, 68-69
 cleaning of, 32
- Pituitary gland, tumor of, 317
- Placenta, retention in bovine, hemograms and bibliography, 330-334
- Plasma cell, immune response, function in, 278
 myelomatosis, canine, 300
 equine, photograph of, in bone marrow, 305
- Plasma protein, in trichostrongylidosis, bovine, 354
- Plasma volume, bibliography, 27
 in hematocrit test, 60
 "trapped" plasma, 18
 methods for measurement of, 17-18
- Plate I, maturation of granulocytic leukocytes, in aspirated bone marrow, canine, 97
- II, maturation of the erythrocytic cells, aspirated bone marrow, canine, 103
- III, Canine blood cells, 109
- IV, Feline blood cells, 113
- V, Bovine blood cells, 117
- VI, Ovine blood cells, 119
- VII, Equine blood cells, 123
- VIII, Porcine blood cells, 127
- IX, the erythrocyte in disease, 247
- X, the leukemia complex, 287
- Plumbism, in the canine, 360
- Poikilocyte or poikilocytosis, 96, 247
 in congenital porphyria, bovine, 361-362
 in haemonchosis, ovine, 230, 354
 in hypersplenism, canine, 366
 in lead poisoning, chronic, canine, 230
 in nephritis, chronic interstitial, canine, 230
 origin, theory of, 229-230
- Polychromasia, polychromatic, polychromatophilia, definition of, 96
 equine, lacking in peripheral blood in erythrogenesis, 122, 347
 in piglets, 125, 191
 photograph of, 247
- Polycythemia, 93, (*See also* Erythrocytosis).
 in Cushing's syndrome, 218
 relative, 236
 vera, 236
 bibliography, 264
 in canine, 236
 in bovine, 236
- Porcine blood, (*See* Pig blood).
- Porphyria, 225
 congenital, in bovine, 226, 361
- Porphyria, 225
- Price-Jones curve of erythrocyte size, 74
 in sheep, 173
- Progranulocyte, description of, 99
 heteroplastic cell, 102
- Prolymphocyte, description of, 100
- Promegakaryocyte, description of, 100
- Promonocyte, description of, 100
- Prorubricyte, description of, 101
- Prothrombin, deficiency of, in canine infectious hepatitis, 323
 ionizing radiation, effect upon, 212
 liver, production by, 212
- Protoporphyrin, 224-225
 in chronic infections, 263
- Pteroylglutamic acid or PGA (folic acid), 252
- Pyometra, bibliography, canine, 333
 canine, case report, 338
 hemograms in, 314
- Pyridoxine, deficiency of, 252
 erythrocyte survival time, in deficiency of, 229
- R
- RABBIT bone marrow, cell characteristics, 209

- Radiation, effects in canine, 261-262
 hematopoiesis, effect upon, 211-212
 immunologic defenses, neutralization of, 212
 in hibernating animals, 262
 lethal dose, various animals, 212
 leukemia, in a hog, following, 285, 308
 leukopenia superimposed, leukocyte response, 275
- Reed-Sternberg cells, in Hodgkin's disease, 282, 283
- Reticulocyte, 96, 101, 220-221, 247
Anaplasma marginale, not parasitized, 221
 bibliography, 232
 "buffy" coat, position in, 60
 characteristics of, compared to the erythrocyte, 221
 crenation, resistant to, 221
 hemolysis, resistant to specific serum, 221
 in bovine, 160
 in canine, 135
 in caprine, 176
 in equine, 180
 in feline, 148
 in health, various domestic animals, 221
 in ovine, 173
 in porcine, 191
 interpretation of occurrence in peripheral blood, 105, 221
 MCV, effect upon, 220
 PCV, position in, 60
 ribonucleic acid content, 220
 ripening time, 220
 rouleaux, failure to participate in, 54, 60, 221
 sedimentation, erythrocytes, effect upon, 57, 60
 size, 220, 221
 splenectomy, effect upon, 214
 staining and counting, 48, 220
 target cell type, 245
 time for release to peripheral blood after blood loss or destruction, 220, 245, 353
 wet blood film method for detection of, 51, 52
- Reticulocytosis, depression anemias, lacking in, examples of 337, 341, 344, 346, 354
 in blood loss or hemolytic disease, 211, 221, 348, 349, 353
 in vitamin B₁₂ and folic acid injections in piglets, 250
 lacking in equine in response to blood loss or destruction, 122, 211, 221, 347
 wet blood film, method for detection of, 51, 53
- Ribonucleic acid, RNA, function in maturation of blood cells, 219-220
- Reticuloendothelial system, 215
 hematopoiesis, function in, 205
 hemoglobin catabolism in, 215
 bilirubin production, 213
 biliverdin production, 226
 hemolytic anemia, function in, 61
 hemosiderin in, 215
- Rouleaux, 52, 96
 animal species, occurrence in, 96, 133
 in bovine, 115, 117, 161, 237
- Rouleaux, animal species, occurrence, in canine, 107
 in caprine, 176
 in equine, 122, 123, 180
 in feline, 112
 in ovine, 121, 173
 in porcine, 125, 127, 195
 in disease, bovine, examples of, 342, 367
 significance of, 115, 237
 leptocytes, participation in, 238, 246
 reticulocytes, failure of participation in, 54, 221
 sedimentation, erythrocytes, relation to, 237
- Rubriblast, description of, 100
- Rubricytes, description of, 101
 hemoglobin synthesis in, 105
 ribonucleic acid in, 219
- S
- SAHLI hemoglobinometers, 66
- Sedimentation rate, erythrocyte, 55-57
 correction chart, Wintrobe, photograph of, 56
 correction table, arranged by PCV value, for canine, 139
 negative value, when corrected, immature erythrocytes, cause of, 348
 in hepatitis, canine infectious, 323, 324
 in hypothyroidism, canine, 319, 346
 leptocytes, a cause of, 362
 meaning of, 139, 238
 diffuse, in hypothyroidism, canine, 346
 diphasic, examples of, 349, 364
 meaning of, 57, 60
 factors affecting, anticoagulants, 34-35, 39, 56
 erythrocyte volume or number, 56, 237
 hemoconcentration, 238, 335, 341
 leptocytosis, 238, 246, 346, 362, 364
 reticulocytosis, 57, 60
 rouleaux or erythrocyte agglutination, 237
 temperature, 56
 in disease, occurrence of, 237-239
 value in equine blood, 237
- Septicemia, degenerative left shift in, 273
- Seromucoid concentration in plasma, measure of inflammatory disease, 161, 173
- Sertoli cell tumor, in canine, 317
 in equine, 339
- Sheep blood, 166-174
 bibliography, 200-210
 cells of, 119-122
 composition of, in lambs compared with adults, 167
 erythrocyte, diameter, 172
 fragility of, 173
 life span of, 229
 sedimentation rate of, 173
 general considerations of, 132, 166-167

- Sheep blood, hemoglobin concentration,
 adult sheep, 172
 effect of altitude upon, 172
 effect of nutrition and parasitism
 upon, 167
 icterus index, 174
 leukemia complex in, 308
 leukocyte counts, total and differential, 173
 neutrophils, toxic changes in disease, 271
 Myeloid-Erythroid ratio, 173, 174
 normal values, 130, 131, 166
 age, in relation to, 171
 from the literature, 168-171
 in parasite-free lambs, 171, 172
 PCV in adult sheep, 172
 reticulocytes, occurrence of, 173
 serumocoid plasma level, estimation of,
 for evidence of inflammation, 173
 sex and breed differences, 172
 specific gravity of, 174
 thrombocytes in, 173
 Shock, from blood loss, canine, 25
 transfusion reaction, a cause of, in canine,
 364
 Simmond's disease, 218
 Spencer hemoglobinometer, 65, 66
 Spherocyte, definition of, 99
 Spherocytosis, hereditary, in man, 259
 in acquired symptomatic hemolytic anemia,
 canine, 357, 359
Spherophorus necrophorus, in bovine liver
 abscesses, 367
 Spleen, anemia, as a cause of, 214, 259, 365
 cirrhosis of, due to excess iron, 215
 erythrocyte, destruction by, 214, 231
 storage of and release in strenuous exer-
 cise, 213
 erythropoiesis, control over in bone marrow,
 214
 in hypersplenism, 366
 granulopoiesis, in hypersplenism, 366
 hypersplenism in the canine, 365
 in anaplasmosis, 213, 231, 257
 in haemobartonellosis, 213, 231
 in hematopoiesis, embryonic period, 206-
 207
 function in post-natal life, 205, 213
 leukemia, basophilic, in feline, effect on, 303
 leukocyte sequestration in, 268
 pulp injections, value of, following lethal
 ionizing radiation, 212
 removal of, effect on blood and bone marrow
 cells, 214
 spherocytes, action on, 231
 stress in feline, contraction of, 143
 target cells, occurrence after removal of, 249
 Splenomegaly, 259, 365
 Staining blood films, basic principles, 43-44
 buffer for, 45
 neutralization of water for, 44
 poor results, causes and corrections, 48
 Stains, brilliant cresyl blue, for reticulocytes,
 48, 96, 101, 220
 for wet blood film, 51
 Giemsa's, 43-44, 48
 new methylene blue, for wet blood mounts,
 51, 53
 Stains, Romanowsky types, 43
 Wright's, 43-44
 modified, 45
 Wright's-Leishman, 47
 Stomach, hematopoiesis, function in, 205
 "intrinsic" factor of Castle, from gastric
 mucosa of swine, 214
 produced by, 214
 Stress, leukocyte responses in, 270-271
 eosinophils, action on, 269
 examples of in the bovine, 162, 272,
 326, 330, 342
 in the canine, 338, 341, 343
 in the equine, 340, 341
 in the porcine, 195
 in various animals, 133
- ## T
- TALLQVIST hemoglobinometer, 66
 Target cell, definition of, 94
 bibliography, 264
 canine, in health, 107
 in blood loss, 245
 occurrence following splenectomy, 214,
 249
 in ligation of splenic vein, 249
 photographs of, 247
 Terminology, blood cells and diseases of the
 blood, fundamentals, 92-99
Theileria mutans, observed in wet blood film,
 54
 Thrombocytes, 100, 205
 azurophil granules in, 91
 "buffy" coat, position in, 59, 366
 embryo, development in, 206
 in blood loss, 244
 in bovine, 116, 165
 in canine, 107, 142
 in equine, 122, 185
 in feline, 112, 150
 in ovine, 121, 173
 in porcine, 125, 196
 origin, in birds, 210
 in mammals, 210
 splenectomy, effect upon, 214
 stress, effect upon in the feline, 143
 vitamin B₁₂ deficiency, reduction in, 210
 wet blood film, examination for, 54
 Thrombocytopenia, blood clot, characteris-
 tics of, in, 81
 "buffy" coat, effect upon, 364
 in hypersplenism, 259
 in poisoning, bracken fern, 260
 trichloroethylene-extracted soy bean
 meal, 260
 in the radiation syndrome, 261
 in vitamin deficiency, B₁₂, 211, 250
 "C" deficiency in the canine, 363
 in wet blood film, detection of, 51
 methods for detection of, 81
 Thyroid gland, disease of, 316-319
 Thyroxin, action on folic acid, 252
 Toxic granulation, neutrophils, 116, 271
 Transfusion of blood, 239-240
 blood volume, effect upon, 15

- Transfusion of blood, in anaplasmosis, 240, 257
 in anemia due to auto-immunization, canine, 359
 in haemobartonellosis, feline, 240
 in "idiopathic" anemias, value of, 240, 350
 in iron deficiency anemia, 243
 in macrocytic anemias, due to maturation arrest, 242, 250
 reaction, in canine, following repeated use of, 364
- Transfusion of leukocyte, 268
- Traumatic reticulitis, bovine, 326-328
 bibliography, 334
 case report of, 342
 hemograms in, 327
- Trichloroethylene-extracted soy bean oil meal poisoning, 260
- Trichostrongylidosis, 264, 354
- Tryptophan, in nicotinic acid synthesis, 251
- U
- UREMIA, leptospirosis, canine, 320, 343
 in nephritis, chronic interstitial, canine, 263, 312, 344, 345
 in pyometra, canine, 315, 316, 338
- Urine, albuminuria, abscess of kidney, canine, 336
 in cellulitis, 338
 in infectious hepatitis, 324
 in leptospirosis, 320
 in nephritis, chronic interstitial, 344, 345
 in peritonitis, 335
 alkaline, dissolves casts, 345
 Bence-Jones protein, absent in equine myelomatosis, 307
 bile pigments, examples of occurrence in 324, 335, 336, 338
 interpretation of, 213, 227
 chlorides, low, indicative of loss by vomiting, 336, 344, 345
 coproporphyrin in, in congenital porphyria, bovine, 362
 indicanuria, indicative of intestinal putrefaction, 335-336
 sediment, casts, examples of occurrence of, 335, 336
 specific gravity of, in Cushing's syndrome, 317
 in nephritis, canine, 312, 344, 345
 uroporphyrin pigment, in congenital porphyria, bovine, 362
 virus of canine infectious hepatitis, presence in, 324
- Uroporphyrin, 225
 deposition in bones and teeth, in congenital porphyria, bovine, 361
- V
- VAN DEN BERGH tests for bilirubin content of plasma, 226-227
- Van den Bergh tests for bilirubin content of plasma, example of in disease, cellulitis canine, 338
- Viral diseases, leukopenia, in, 274
- Vitamins, 249-253
 ascorbic acid or "C", 252
 conversion of folic acid to folinic acid, 252
 deficiency of, in canine, a case report, 362-364
 in iron absorption, 254
 B₁₂, 249-251
 "animal protein factor" relationship to 250
 in cell mitosis, essential for, 219
 deficiency of, cell morphology, in blood and bone marrow, 210-211
 cobalt, relationship to, in ruminants, 251
 macrocytic anemia, typical of, 242, 250
 megaloblastic bone marrow, characteristic of, 95, 250
 equines, source of, 251
 erythropoiesis, essential for, 222-223
 function of, 210
 liver storage of, 205, 212
 rumen synthesis and cobalt requirements, 222, 251
 stomach, importance in man, 214
 "intrinsic" factor, relationship to pernicious anemia, 214
 synthesis, cecum and feces, by bacterial action, 251
 folic acid, 250-252
 antimetabolites for, in treatment of leukemia, 252
 deficiency, erythrocyte survival time in, 229
 in canine, 251
 megaloblasts in bone marrow, 95
 folinic acid or citrovorum factor, active form of, 252
 function of, 210
 in macrocytic anemia, 242
 liver storage of, 205, 212
 mitosis, essential for, 219
 para-amino-benzoic acid, relationship to molecule of, 252
 PGA or pteroylglutamic acid, chemical name for, 252
 synthesis, in the canine, 251
 nicotinic acid relationship to, 251
 thyroxin, action on, 252
 niacin or nicotinic acid, 223, 242
 folic acid, relationship to in canine, 251
 tryptophan, relationship to, 251
 pyridoxine or B₆, 223
 in iron utilization, 252
 riboflavin or B₂, 223
 thiamin, 223
- W
- WATER balance studies, 22-24
- Wright stain, 43-44, 45

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